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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

U.S. National Cancer Institute
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SUMMARY REPORT

TUMOR IMMUNOLOGY PROGRAM

Fiscal Year 1978

An immunology program has been designed which has as its goals (1) the use of immunologic techniques to detect agents etiologic for cancer, (2) the prevention of cancer by immunologic manipulations, (3) the use of immunologic techniques to improve the diagnosis of cancer, and (4) the manipulation of the immune system of patients with established cancer so that increased survival is achieved.

The program has four operational components (designated Immunobiology, Immunodiagnosis, Immunology of Cancer Cause and Prevention, and Immunotherapy) and each program area has a number of currently funded projects.

A. IMMUNOBIOLOGY

The Immunobiology Program area supports research in immunology that may have relevance to cancer.

Natural killer cells (NK) seem to be a significant cell group with regard to tumor resistance in vivo. Interferon and interferon inducers including Cornynebacterium parvum seem to be major activating agents for natural killer cells in vivo (Wigzell). Murine and NK cells have a certain cross reactivity but show a better fit for targets of their own species (Klein). The effect in vitro of leukemia-associated immunosuppressive cells (found in the spleens of leukemic mice) can be reversed by increasing the Ca^{++} concentration in the media, suggesting that immunosuppressive cells influence the cyclic nucleotide levels in the responder cells (Cerny). In studies on mechanisms whereby activated macrophages kill tumor cells, it has been shown that inhibition of cytotoxic effect roughly parallels inhibition of protein synthesis (Churchill). Studies on antitumor effector cells of mice show that, in addition to being present in the peritoneal cavities of tumor-immune mice, they are also present in the peritoneal exudates induced in normal mice by protease peptone, or following infection with Listeria, Salmonella, or BCG (Nelson). A systematic study of the effect of mixed lymphocyte and Con A stimulated subpopulations on the cytotoxic responses of unstimulated subpopulations of T cells to allogeneic and syngeneic tumor cells is being done (Stout). Different tumors studied have varied in their susceptibility to natural cytotoxic cells and the susceptibility correlated inversely with their ability to grow in T cell deficient nude mice; the activity appeared not to be mediated by macrophages, eosinophil granulocytes, or lymphocytes but was particularly strong in polymorph-rich cell populations obtained from peritoneal fluid (Nossal). A system is being developed to delineate the contribution of tumor specific T cells to immunity against tumor in vivo (Shen).

Studies on mechanisms of T cell mediated cytotoxicity at the molecular level using spin labelling to study cell interactions are being done (Whisnaut). Attempts are being made to elucidate the chemical nature of specific cell surface receptors involved in the interaction between cytotoxic T cells and target cells (Berke). It has been shown that IgG induced inhibition of human K cell activity results from the redistribution of Fc receptors on the K cell surface (Henney). Another study has provided insight into the regulatory cellular events necessary to generate an immune response to tumor cells in man (Schlossman). Immunogenicity and susceptibility of tumor cells to cytotoxic lymphocytes vary according to their growth phase and density; human spleen cells are a better source for generation of cytotoxic lymphocytes than peripheral blood lymphocytes (Feldman).

Several contractors have collected "non-immunogenic" tumors and studies are underway to determine the role of several processes that might interfere with tumor rejection immunity (Bartlett, Marquet, Embleton).

Efforts are underway to produce continuously propagatable lines of antigen-specific T cell subclasses that each express a distinct immune function (Cantor). The ability of thymus epithelium to induce T lymphocyte differentiation in high leukemic strains of mice is being studied (Waksal). It has been shown that normal thymocytes produce lymphoma when cultured on a monolayer of thymus epithelial reticulum cells from leukemic mice (Haas).

Cell surface changes on highly metastatic rat mammary adenocarcinoma lines and their clones are being studied by immunological, biochemical and morphological means to determine surface characteristics important in mammary tumor metastasis (Nicolson). Cytotoxic lymphocytes generated against monolayers of metastatic pulmonary carcinoma suppress the formation of lung metastases when inoculated with tumor cells from local or metastatic growth, while cytotoxic cells generated against cells of the local tumor are unable to inhibit formation of lung metastases (Segal).

There is strong homology between H-2 and the major histocompatibility complex of other species including man. In the mouse, genes regulating the susceptibility or resistance to tumors reside in the H-2 complex; resistance and susceptibility are co-dominant with heterozygotes displaying an intermediate level of susceptibility (Haughton). In comparing results with three different leukemia virus variants, H-2 linked resistance was found in all three but the resistant and sensitive alleles and loci were different from each (Haran-Ghera).

Evidence has been obtained indicating that lymphoid stem cells are primarily derived from an intra-embryonic source rather than from the yolk sac in studies done utilizing yolk sac-embryo chimeras with sex chromosomes and IgG allotypes are markers (Toivanen). A simple new method for monitoring hemopoietic stem cells, possibly a special subtype, has been developed and has many applications in marrow transplantation studies (Metcalf). In addition, a selective culture system to discriminate between transplanted normal and neoplastic B lymphocytes in the mouse was developed (Metcalf).

It appears that major changes occur in lateral diffusion of both membrane proteins and membrane lipids after activation of a cell by normal fertilization (Edidin).

Anti-idiotype antisera have been developed against AKR responder T-cell blasts for a variety of allogeneic stimulators and TNP modified AKR cells; these antibodies can be used to specifically remove receptor bearing AKR-cells (Krammer). New procedures for detection of antigen-binding T cells are being evaluated (Goodman) with intent to purify T-cell derived antigen-binding receptors. Monospecific anti-idiotypic antisera are being prepared and continuous cell lines of rat lymphoid cells which secrete homogeneous antibody reactive with rat alloantigens are being developed (Fitch). Idiotypic antibody on T cells has been demonstrated to be functionally relevant (Olavi-Makela).

New congenic mouse strains that permit controlled studies on effects of spontaneous autoimmunity on tumorigenesis are being developed and maintained (Murphy). New recombinant and mutant H-2 haplotypes, particularly variants of the I region, are being developed (Shreffler). Immune related cell lines are maintained and supplied on demand (Cohn). An automated system for HLA typing has been refined and is being evaluated (Grumet).

B. IMMUNODIAGNOSIS

The fundamental goal of the Immunodiagnosis Contract Program is to develop and evaluate tests which will be useful for the detection and diagnosis of cancer, and for indicating the prognosis of cancer patients and detecting early recurrences of disease. To accomplish this goal, a multifaceted program has been developed. It includes:

(1) The development and maintenance of resources to support research in immunodiagnosis and to rapidly evaluate the possible clinical usefulness of tests which appear to be able to discriminate cancer from other conditions;

(2) The support of research on circulating tumor antigens, with development of sensitive methods for their detection;

(3) The support of research on immune responses to tumor antigens, which might have diagnostic applications.

A major portion of the Immunodiagnosis Program has continued to be centered around the resources area. Important resources for immunodiagnostic research have been continued and some new resources are now being developed. This particular aspect of the Immunodiagnosis Program is particularly well-suited to contract research and probably could not be satisfactorily accomplished by other mechanisms. The maintenance of a Serum Bank with the Mayo Clinic (Schutt) has been invaluable for the rapid evaluation of potentially useful immunodiagnostic and other serum diagnostic tests. As in the past, during the past year there have been a large number of potentially useful diagnostic tests evaluated by this procedure. It has been possible to accommodate most requests from investigators with preliminary indications of the ability to discriminate between the sera of cancer patients and controls. After the tests on coded serum samples have been completed, the results have been analyzed and correlated with clinical diagnosis and extent of disease. Much assistance with the statistical analysis has been provided by Dr. Mitchell Gail, Biometry Branch, NCI and by Mr. Michael Stump and Ms. Altamese Gales in the ADP Management Section, NCI. As support for these analyses, a statistical support contract was set up last year (Horn) which has been very useful in allowing a rapid analysis and return of information to investigators. Promising results obtained in these serum panels have frequently provided the basis for evaluation of applications for contract support from some of the investigators.

In anticipation of the identification of tests which appear promising for detection of cancer in a high risk population, in 1973, the Committee on Cancer Immunodiagnosis initiated two (2) contracts for the collection of serial serum specimens and clinical information from high risk, screened populations (Altschuler and Najarian). One of these projects (Altschuler) has performed very well and will continue to follow patients for at least an additional one to two years. The other project has had some difficulties in obtaining adequate followup on the screened individuals and will soon be terminated.

In addition to the above serum collections, last year a decision was made to obtain collections of serial specimens from carefullly followed cancer patients after diagnosis (Feit and Gerner). These collections should be quite valuable in the evaluation of serum tests which show some promise for monitoring cancer patients and for detecting early recurrences. In addition, another project (Lynch) has been set up to obtain large volumes of plasma by plasmaphoresis from known cancer patients and controls. These specimens will be used to set up standard panels of specimens from patients for comparative studies amongst various serologic assays. These specimens should also be quite valuable for purification of markers in the circulation.

The other project in the resources area involves the maintenance of a large bank of well-characterized tissue culture cell lines derived from human cancers (Fogh). The purpose of this contract is to provide to investigators engaged in diagnostic research cell lines which may be used as standard sources of tumor antigen. The availability of the cultured cell lines has been widely advertised in cancer diagnosis research. The Bank now includes 401 human tumor cell lines. These lines are being extensively evaluated in regard to their characteristics and growth in vitro and production of tumors in nude mice. Isoenzymes and chromosomal analyses have been very helpful in identifying likely cellular contaminants, either with Hela cell lines.

Among a large number of projects related to circulating tumor antigens there are several devoted to studies on the clinical usefulness of CEA and related antigens. CEA is the one immunodiagnostic test which is licensed for general use and is thus widely used. However, there is still sufficient information as to its value for various clinical applications and some of the contracts were designed to determine the usefulness of CEA in various settings. One project which has just been completed (Hersh) was designed to determine the usefulness of CEA as an adjunct in the differential diagnosis of gastrointestinal cancer. This study has provided a rather clear indication that there is considerable overlap between the CEA values of patients with malignant and benign diagnoses and that this assay would probably would not be of much value in differential diagnosis. In addition, this project provided specimens to another contractor (Nakamura) for evaluation of the differential diagnostic usefulness of CEA-S and has indicated that with the current form of the assay, there is not sufficient sensitivity or discriminatory ability. Another project has been concerned with the usefulness of CEA for monitoring cancer patients for prognosis and for early detection of recurrent disease (Chu). The study has indicated that preoperative CEA values in patients with colorectal cancer was a significant indicator of disease recurrence and the combination of CEA and Duke's classification was more significant as prognostic indicators than either alone. The project (Nakamura) on a variant of CEA, termed CEA-S has revealed that the recent results have not provided the additional benefits seen in preliminary tests. Efforts are now being made to determine what factors have caused the decrease in discriminatory ability.

Fetal sulphoglycoprotein in gastric juice has been shown to have promise in screening for gastric cancer. The Program is supporting a project (Hakkinen) to determine more precisely the value of this test for early detection of gastric cancer, and to develop a more sensitive assay. The results thus far are promising but there have been a number of cancers developing in the antigen-negative individuals. Further followup will be necessary to determine the practical value of this test.

The Immunodiagnosis Program has supported a number of projects for development of new human tumor-associated antigens which might be useful in immunodiagnosis, particularly those which would circulate and thereby form the basis for serum diagnostic tests. One project for detection of acidphosphatase by radioimmunoassay showed promise in initial tests on a coded serum panel and a prospective trial for its diagnostic usefulness is now underway (Cooper). A number of antigens associated with various types of cancer are now being characterized and attempts are being made to develop sensitive assays for examination of circulating levels. An emphasis has been placed on studies of antigens associated with the more common tumor types: gastrointestinal cancer (Saravis), cervical cancer (Alexander and Ibrahim), ovarian cancer (Lloyd), lung cancer (Veltri), melanoma (Lloyd) and leukemias (Chiu, Humphreys and Minowada). Because of indications of cross-reacting antigens between human tumors and microbial agents, a project was set up to determine the diagnostic usefulness of this approach (Granatek). A project for measuring copper and ceruloplasmin levels in serum (Linder) has indicated some prognostic usefulness in lung cancer. Another project (Chiu) has attempted to produce antibodies against non-histone nuclear proteins in various histologic types of cancer.

Detection to antibodies to tumor-associated antigens has great potential for early detection of tumors. The Immunodiagnosis Program is supporting two projects utilizing animal models to develop more sensitive procedures for antibody detection (Witz and Stutman). In the latter project, a study is now underway to document the usefulness of antibodies mediating cell-mediated cytotoxicity for early prediction of tumor appearance. A similar project looking for antibodies to human melanoma by antibody dependent cell mediated cytotoxicity has recently been initiated (Hersey).

Cancer patients who produce antibodies against circulating antigens may have circulating antigen antibody complexes which are detectable by several sensitive assays which have been developed (Theofilopoulos and Schrohenloher). These assays are being evaluated for their usefulness in monitoring cancer patients and sera with high levels of complexes are being analyzed to determine the nature of the antigens in the complexes. This approach may be useful in identifying new tumor associated antigens which are immunogenic to the host. An animal model in this area, looking at circulating complexes in the sera of cats with feline leukemia is also being performed (Burger). This project has indicated that many of the immune complexes detected involve antigens unrelated to feline leukemia.

The last major research area being supported by the Immunodiagnosis Program is concerned with cells involved in immune responses and particularly in cell mediated immunity to human tumor antigens. To facilitate studies in this area, it has been thought advisable to develop better methods for identification and isolation of effector cells that might be involved in these reactions. Two projects have focused on methods for separating subpopulations of blood lymphocytes (Schlossman and Wigzell). Both of these projects have provided much important new information on markers for lymphocyte subpopulations and on methods for their physical separation. A new project was initiated to evaluate the usefulness of receptors for lectins on subpopulations on human lymphocytes (Sharon). Receptors for peanut agglutinin may be useful in the classification of acute leukemia. Another project (Talmage) has been devoted to isolation of peripheral blood monocytes in high yield and purity and this has been quite successful. In order to standardize the assays for cellular immunity and to facilitate the exchange of cells amongst laboratories, cryopreservation of functionally active cells is quite important and two projects (Weiner and Loos) have been set up to develop practical methods for cryopreservation of human monocytes. Two other studies have been initiated to examine the abnormal functional properties of monocytes in cancer patients (Snyderman and Berlinger). The former project has already provided indications that abnormal response of monocytes to chemotactic factor is a poor prognostic sign. This project has also provided some informaion on the nature of a soluble material which inhibits chemotaxis. The latter project (Berlinger) has focused on the effects of suppressor monocytes in cancer patients and the relationship between these cells and course of disease.

A project on improvement in methods for detection of cell mediated immunity will be completed shortly (Thor). This project involved the development of the microagarose droplet method for measuring migration inhibition of macrophages and leukocytes. This study indicated the presence of specific cellular immunity in some bladder cancer patients against common tumor associated antigens. Several other projects have utilized the assays for cellular immunity, both in vivo and in vitro, for identification and isolation of human tumor associated (Reisfeld, Malley and Wright). Two of these projects have had some success in preparing heterol antibodies to isolated melanoma associated antigens and efforts are now being made to develop radioimmunoassays. In the third project (Wright) progress has been made in the separation of kidney cancer associated antigens. An addional project in this area has recently been initiated (Oldham) for purification of breast tumor associated antigens with subsequent development of radioimmunoassay for those antigens. Over the last few years, much attention has been directed toward cell mediated cytotoxicity of cancer patients against human tumor cell lines. A major problem in this area of research has been the occurrence of cytotoxicity by normal individuals against these cell lines. Two recently initiated projects (Takasugi and Oldham) are designed to carefully study the phenomenom of natural cytotoxicity and to find ways to distinguish this from specifically immune reactivity of cancer patients. A further project in this area has focused on the possible augmentation of the specific disease related cytotoxicity by culturing with autologous tumor cells (Shiku).

One procedure for measuring cellular immunity had been claimed to be useful for diagnosis of cancer. Two projects are just being completed which evaluated the usefulness of this test, the macrophage electrophoretic mobility test (Pritchard and Weiss). In both of these studies, the test was shown not to be sufficiently discriminatory for diagnostic purposes. Efforts are being made in both of these laboratories to improve the discriminatory ability of the procedure.

As in the past, the most important accomplishments of the Immunodiagnosis Program have been the maintenance of important resources for development and rapid evaluation of potentially useful immunodiagnostic tests. With the extensive use of the Serum Bank, it has been possible to identify tests with promise for immunodiagnosis and also to identify tests, which had previously been thought to be valuable but which actually could not adequately discriminate. These efforts have had major impact on the course of immunodiagnosis research.

C. IMMUNOLOGY OF CANCER CAUSE AND PREVENTION

The goals of the Immunology of Cancer Cause and Prevention Program are to develop immunologic methods that may be useful in experimental studies on the etiology and prophylaxis of cancer. Means are being sought to (1) detect carcinogens in the environment and in body fluids and organs, (2) uncover and correct possible immunologic defects accompanying carcinogenesis, and (3) inhibit carcinogen-induced cell transformation and premalignant neoplasia.

1. Detect Carcinogens in the Environment and in Body Fluids and Organs - In previous contract-supported research projects, it was found that the ability of guinea pig macrophages to synthesize certain components of complement (C2 & C4) was impaired if the cells were obtained from animals treated with the chemical carcinogen dimethylnitrosamine. The current contractor (Strunk) has confirmed this observation but has

found that none of ten additional chemical carcinogens inhibits the synthesis of both C2 and C4. Aflatoxin B_1 and benzidine inhibited C2 synthesis and $BeSO_4$ inhibited the synthesis of C4. This contract is being terminated.

Radioimmunoassays have been developed for the detection of picomoles of nicotine, some of its metabolites and analogues, polycyclic aromatic hydrocarbons, mycotoxins, polychlorinated insecticides and anti-neoplastic agents (Van Vunakis). These assays may be useful in basic studies of chemical carcinogenesis and in applications such as the detection of noxious agents in body fluids.

2. Uncover and Correct Possible Immunologic Defects Accompanying Carcinogenesis - A study based on the observation that some chemical carcinogens are also contact allergens is being terminated but has continued without additional funds (Pomerance). Guinea pigs were first made immunologically unresponsive to chemical carcinogens and susceptibility of these animals to tumorigenesis by the corresponding carcinogen was compared to that of animals sensitized to the agent. These and untreated animals did not differ significantly in the rate and extent of chemical carcinogen-induced tumor formation. Breast tumors appeared in strain 2 guinea pigs after intravenous administration of dimethylbenzanthracene. These tumors have not metastasized; transplants of breast tumor tissue into healthy, syngeneic recipients have failed to grow. Two projects that are being terminated (Zwilling and Osler) have been continued without additional funds. These two projects are devoted to studies of the effects of chronic administration of chemical carcinogens to animals on various aspects of immunity. So far immune reactivity, based on the results of a variety of tests, has been unchanged or only minimally affected in carcinogen-treated animals. Changes that have been observed occur when neoplasia is evident. It was concluded that chronic administration of doses of a chemical carcinogen that produce tumor in a significant number of animals caused no detectable immunologic defects at any stage in the carcinogenic process preceding tumor formation.

3. Inhibit Tumorigenesis Prophylactically - A group of projects (Goren, Gensler, Clark, Cockerell, Lasfargues, Kreider, and Olsen) is directed toward the use of BCG or its subfractions as a possible means of interrupting carcinogenesis before the development of cancer.

a. It is known that trehalose dimycolate, a fairly well-defined fraction of BCG, is partly responsible for the anti-tumor action of this mycobacterial immunoadjuvant. This compound as well as related structures of mycobacteria that have been isolated and characterized will be tested by various investigators for immunoprophylactic anti-cancer activity (Goren). This contract is being terminated.

b. An attempt is being made to totally synthesize trehalose dimycolate (Gensler). An intermediate product has been synthesized and work is proceeding to the final product. This contract is being terminated.

c. Primary malignant melanoma, pigmented and metastatic, has been induced in allogeneic guinea pigs by chronic, topical administration of methylbenzanthracene. Preliminary studies indicate that injection of a mycobacterial vaccine before the development of premalignant lesions or directly into premalignant lesions inhibits the progression of carcinogenesis (Clark). This contract is being terminated.

d. N-methyl-nitrosourea will be administered to outbred guinea pigs by intrarectal instillation. Most of the two-year delay in the initiation of carcinogen administration has been due to the design and installation of safety equipment conforming to the demands of the biohazards committee of Cornell University. The original principal investigator, Dr. Andrews, has resigned from the University. The current principal investigator is Dr. Gary Cockerell who is responsible for almost all of the progress that has been made since the start of the project. Emulsified BCG cell walls have been injected, subserosally, into the descending colon of normal guinea pigs. Although vaccine administered in this manner produces severe focal, transmural granulomatous colitis which eventually resolves, the animals remain completely asymptomatic. Surgical technique for tumor resection has been perfected. The only successful form of surgery of the lower bowel in guinea pigs has been a diverting colostomy.

e. Extracts of cells derived from mouse mammary tumor lines will be tested as vaccines for the immunoprophylaxis of primary endogenous murine breast carcinoma. Experiments are under way testing routes and doses of inocula and the influence of immunoadjuvants on the effectiveness of specific vaccines (Lasfargues).

f. Immunoadjuvants and specific vaccines will be used to treat rabbits with naturally-occurring Shope papillomas in attempts to prevent progression from the benign to malignant disease. Intratumoral injections of BCG cell walls on oil droplets has produced extensive destruction of papillomas but no apparent inhibitory effect on progression of disease. Vaccination with allogeneic papilloma tissue shows promise as a means of inducing immunity against challenge papillomas (Kreider). Evaluation of results ultimately will be based on the incidence of carcinomas in treated animals.

g. Cats infected with feline leukemia virus will be studied to determine the presence and distribution of feline oncornavirus-associated cell membrane antigen (FOCMA) in various tissue and organs. Immunofluorescence studies of tissues of cats infected with feline leukemia virus revealed the presence of group specific antigens in intestinal epithelium, bone marrow, spleen and lymph nodes. FOCMA has been detected in bone marrow and in lymph nodes at least six months before tumor formation.

D. IMMUNOTHERAPY

The goals of the Immunotherapy Program are to (1) identify immunologically active agents that lead to cure, regression or stabilization of established tumors; (2) determine the mechanisms whereby such agents work; and (3) assess the effectiveness of these agents in patients with cancer. Evaluation of immunologically active agents is performed in animals bearing tumors that have some resemblance to human tumors, or that are already known to be responsive to immunologic manipulations. Once suitable tumor systems are identified, agents known to be immunologically active can be optimized and/or compared for relative efficacy, and agents that are suspected of being active can be tested. MER, Levamisole, and killed BCG have been evaluated extensively in mouse, rat and guinea pig tumors with variable and differential activities obtained but neither cure nor striking prolongation of survival observed (Bartlett). In combination with systemic chemotherapy, intratumoral (but not systemic) injection of C. parvum in rats and in mice led to prolonged survival or cure (Bartlett, Fisher), and C. parvum alone to control of metastatic spread in rats (Baldwin). C. parvum also enhances the effectiveness of specific xenoantisera in prolonging the survival of mice with ovarian carcinoma and a corresponding synergistic response was observed in vitro (Bast). BCG and C. parvum cause little or no suppression of a guinea pig leukemia (Murphy).

In vitro activated T cells can cause tumor regression in vivo. Improvements have been made in the in vitro sensitization of lymphoid cells against tumor cells and their antitumor activity assessed (Kaplan, Kedar, Palmer). Aiming at the possibility of large scale production, studies are in progress on the evaluation of continuous culture methods for tumor specific cytotoxic lymphocytes (Smith).

Spontaneously occurring leukemias and lymphomas in outbred cats provide an important model and studies of passive and active immunotherapy are being carried out (Essex). Also, in progress, are studies of the induction of specific immunity to transplantable and primary fibrosarcomas in inbred chickens (Lerman).

A variety of newer approaches are being supported. Neuraminidase modified plasma membranes in combination with chemotherapy increase the survival period of tumor bearing mice (Pincus). Tumor cells have shown antigenic changes after being exposed to chemotherapeutic agents. The use of lymphocytes sensitized to the modified tumor cells in therapy against the parent tumors is under study (Bonmassar). Individual tumors have been shown to be heterogeneous with respect to cell surface carbohydrates. Study of the effectiveness of subpopulations as immunotherapeutics in leukemic mice has given variable results (Killion).

Other approaches under investigation include the conjugation of chemotherapeutic agents to antitumor antibodies to learn whether this improves chemotherapeutic effects (Arnon), inducing thyroiditis in rats to determine whether the induction of immunity to an organ can lead to destruction of a tumor originating in that organ (LoGerfo), studying the ability of tumor necrosis factor to destroy established murine tumors in vivo (Old), using immune RNA and transfer factor in primates (Eichberg) and determining whether lymphokine preparations will induce regression in animal tumors (Papermaster).

Macrophages are believed to play an important role in many immune interactions. In vitro non-specifically activated macrophages are studied for immunotherapeutic activity (Piessens). The functional characteristics of macrophages from normal and tumor bearing animals have been compared (Schmidtke) and assays for human monocyte functions have been developed and applied to studies in cancer patients (Schmidtke, David, King). Antigens, antigen-antibody complexes and effector cells may be involved in the generation and/or suppression of immune responses to tumors. These substances are being isolated and tested for their effect on a rat tumor (Feldman).

Naturally occurring tumors often are poorly antigenic. A variety of studies are ongoing in an effort to increase immune responsiveness to tumor cells. Hybrid cells appear to lose oncogenic potential but retain the surface characteristics of neoplastic cells. Studies are in progress to learn whether such cells provide therapeutic benefit in murine leukemia (Cohen). Further, in an effort to increase immune response to tumors, immunization is being studied with haptenated tumor cells (Hamaoka). Selective destruction of neoplastic cells follows infusion of serum, plasma or whole blood. Plasma, concentrated for antileukemic activity will be tested as an adjunct to chemotherapy in leukemic mice (Kassel).

Increase in cell-membrane microviscosity increases membrane immunogenicity. Studies are being carried out to increase the immunogenicity of mouse tumor cells through alteration of membrane microviscosity with the ultimate aim of applying such information in immunotherapy (Schinitzky).

Central to the application of immunology in cancer therapy is the existence and the role of tumor associated antigens in host resistance to tumors. Studies are in progress to demonstrate the existence of such specific antigens in human tumors and to elucidate the biology of immunologic response (Klein).

The clinical part of the immunotherapy program has included a number of studies designed to determine maximum tolerable dosages and also evidence for an effect on the immune system by agents that are believed to be immunologically active. The agents examined include C. parvum and levamisole (Hirshaut), several BCG preparations (O'Connell) mycobacterium phlei cell wall skeleton + P3 in oil in water emulsion (Vosika) irradiated and neuraminidase treated leukemia cells (Zighelboim) and BCG plus irradiated melanoma cells (Oettgen). Studies are in progress on the categorization of renal carcinoma antigens and on the effects of immunization with allogeneic carcinoma cells plus BCG on antigen levels (Oettgen).

Clinical trials to determine the effectiveness of immunotherapeutic agents have yielded promising results in lung cancer. The introduction of living

BCG into the pleural space after resection of lung cancer has significantly increased the disease free interval and survival of patients with stage I disease when compared to randomly selected control patients treated with surgery alone (McKneally). Other immunotherapeutic trials in patients rendered free of disease by surgery include the use of soluble, partially purified extracts of lung cancer membranes mixed with Freund's adjuvant (Takita), the use of MER postoperatively in patients with squamous cell carcinoma of the lung (Cortes), treatment with levamisole in patients postoperatively for squamous cell cancer of the head and neck (Strong), specific plus nonspecific immunotherapy as an adjunct to chemotherapy, post excision, in skeletal and soft tissue sarcomas (Eilber) and BCG with or without tumor cell vaccine after surgery in melanoma (Morton).

Vibrio-cholera-neuraminidase treated allogeneic blast cells are being evaluated for therapeutic benefit in patients on maintenance chemotherapy with acute myelogenous leukemia (Holland); the further addition of MER provides no benefit and has been dropped from the study (Holland). "Unblocking" plasma was found to be without benefit in malignant melanoma (Hellstrom). Patients with more advanced stages of cancer are being treated with many of these and related agents (Hersh and Fahey). The addition of C. parvum to chemotherapy in patients with disseminated breast cancer, once thought to prolong survival, is now regarded to have questionable benefit (Pinsky).

Radiotherapy causes strong immunosuppression. Studies are being carried out to document and analyze such suppression and to evaluate immunostimulating agents in countering such suppression. Immunostimulants under study are thymosin (Wara) and BCG plus levamisole (McLaren).

Animal studies have suggested that immunostimulants such as BCG may be more effective therapeutically if given before tumor removal. Studies are being carried out with intratumoral administration of BCG in patients prior to radiation and cystectomy in bladder cancer (Pinsky) and prior to surgery in lung carcinoma (Matthay). Randomized prospective studies have been initiated to confirm favorable preliminary reports on the use of BCG in recurrent superficial bladder cancer (Lamm and Pinsky).

Also supported was work of general benefit to the field of immunotherapy, including the development of a new standardized skin test device (Hein) and the identification of inbred rabbit strains that produce high levels of complement activity (Cherry).

The outstanding accomplishment in work supported by this program has been the study of McKneally suggesting that intrapleural BCG can prolong survival of patients with stage I lung cancer.

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator:	Dr. Robert Stout
Name/Address	Brandeis University
Performing Organization:	Rosenstiel Research Center
	Waltham, MA

Contract Number: N01-CB-64034
Starting Date: 6/15/76 Expiration Date: 6/30/78

Goal: To develop a system for stimulation of isolated amplifier T cells so as to allow amplification of cytotoxic responses to syngeneic cells which bear tumor-specific antigens but no allogeneic Ia antigens.

Approach: The approach entails a systematic study of the effect of mixed lymphocyte and Con A-stimulated subpopulations on the cytotoxic responses of unstimulated subpopulations of T cells to allogeneic and syngeneic tumor cells. The subpopulations of T cells will be detected by the presence of surface Ly or FcR determinants and isolated on a Fluorescence Activated Cell Sorter.

Progress: All required culture and assay systems have been established and key reagents have been prepared and tested. Conditions for optimal stimulation of MLR and CML responses in microculture have been determined. MLR proliferative responses of 2 x 10^5 responder lymphocytes increased as the number of stimulator cells was increased 16 x 10^5 cells. Lymphocytic responses in similar cultures increased as the number of stimulators was increased to 4 x 10^5 cells and decreased thereafter. The ability to isolate the interacting T cell populations for CML responses in the B6 mouse system on the basis of the presence or absence of the FcR (Fc receptor) was confirmed. Optimal CML was generated only when both FcR negative and FcR positive responder T cells were present in culture. MLR activated T cells can, when added to fresh cultures, increase the subsequent CML response. This increase ranged from 1.5 to 4-fold in different experiments when 1 x 10^5 MLR-activated cells were added to 4 x 10^5 fresh responder cells. Irradiated MLR-activated T cells can also function in this manner, but culture supernates from MLR cultures do not seem to contain an enhancing factor.

Significance to Cancer Research: NCP Objective 6 Approach 4.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $37,462

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of T Cell-Mediated Cytotoxicity

Principal Investigator:	Dr. Carol C. Whisnant
Name/Address	Duke University
Performing Organization:	Durham, NC

Contract Number: N01-CB-74152
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Study mechanisms of T cell-mediated cytotoxicity at the molecular level.

Approach: Use as a model system the specific killing of allogeneic tumor cells by immune peritoneal exudate cells (CHY and EL-4 tumor cell lines). Spin labeling will be applied to studying cell interactions.

Progress: The tumors EL-4 and CHY, both leukemias of C57BL mice, differ considerably in their susceptibility to lysis both by antibody and complement and by cytotoxic T lymphocytes (CTL). This comparison has been extended to include susceptibility to lysis by human peripheral blood lymphocytes (PBL) in the presence of antibody (ADCC) and by human PBL alone ("natural killing" - NK). CHY is less susceptible than EL-4 to lysis by antibody plus complement, by CTL, and by ADCC, while CHY is more sensitive than EL-4 to "natural killing." From such comparisons we might identify similarities and differences among various lytic mechanisms, establish the relationship between effector cells in ADCC and NK, and explore the "specificity" of NK. CHY originated and is maintained in C57BL Springville mice, while our EL-4 is maintained in C57BL/6 mice. To assess the importance of "environmental" conditions on susceptibility to lysis, we are carrying a line of CHY in C57BL/6 mice, and both CHY and EL-4 have been maintained in tissue culture for several weeks. Preliminary results indicate that the differential susceptibility to lysis persists when CHY is carried in C57BL/6 mice.

Significance to Cancer Research: Elucidation at the molecular level of mechanisms of cell-mediated cytotoxicity may suggest reasons that some tumors are resistant to such effector function and may facilitate manipulation and modification of these processes.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 56,024

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Jan Cerny
Name/Address Harvard College
Performing Organization: Cambridge, MA

Contract Number: N01-CB-64035
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To elucidate the nature, properties, and mode of action of immunosuppressive cells (IsC) which appear in the lymphoid organs of tumor-bearing mice and which actively inhibit the functions of immunocompetent cells in the organism.

Approach: The principal experiments are done by mixing cells *in vitro*. Thus, cells from various organs of mice with malignant tumors are added into cultures of normal immunocompetent lymphocytes (i.e, responder cells) which are being stimulated with an antigen. A diminution of the response indicates the presence of active IsC in the given organ of the tumor-bearing donor. This system allows various manipulations of IsC, prior to their addition into responder cell cultures, in order to elucidate their functional properties.

Progress: Results prior to the present contract year showed that there are immuno-suppressive cells (IsC) in the spleen of leukemic mice. These cells appear to be normal lymphocytes, distinct from leukemia cells. They are Ig-, theta-, and they suppress the immunocompetent cells *via* cell-to-cell contact. During the present year, we confirmed that the effect of the leukemia-associated ISC *in vitro* be reversed by increasing the Ca^{++} concentration in the media; this suggests that IsC influence the cyclic nucleotides levels in the responder cells. We also found that IsC inhibit only those lymphocytes sharing K end of H-2 complex, and that they supress the T-dependent antibody response, preferentially if not exclusively. Exciting preliminary results showed that the suppression of T-dependent response by IsC was abolished by addition of a helper factor. Our current research effort focuses on the possibility of reversing the immunosuppression *in vivo*.

Significance to Cancer Research: Immunosuppressive cells are now being found also in patients with tumors. It is likely that these cells represent one of the reasons why the organism is unable to mount effective immune response to the tumor as well as to various infections. The mouse model permits the analysis of this problem.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $73,664

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-Binding T-Cell Receptors

Principal Investigator:	Dr. P. H. Krammer
Name/Address	Institute for Immunology
Performing Organization:	Heidelberg, Germany

Contract Number: N01-CB-74179
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To test for the expression of alloantigen receptors on murine T-cell tumors.

Approach: Use AKR/J lymphomas; develop sensitive assay systems for alloantigen binding T-cell receptors; obtain sufficient quantities of receptor material from positive tumors to perform biochemical analyses.

Progress: Murine responder T-cell blasts activated by allogeneic stimulator cells have a specific receptor for histocompatibility antigens of the stimulator strain. Receptors on responder blasts have been detected by binding of stimulator cell material in MLR supernatants and by anti-receptor antibodies (anti-idiotypic antibodies = anti-id). We have developed anti-id sera against AKR responder T-cell blasts for a variety of allogenic stimulators and TNP modified AKR cells. These antibodies can be used to specifically remove receptor bearing AKR-cells. Furthermore, we are able to detect T-cell receptors for alloantigens on AKR T-cells by binding of radioactive membrane vesicles from stimulator cells prepared by nitrogen cavitation. These test systems will now be applied to test our collection of 500 AKR T-cell tumors for receptor expression.

Significance to Cancer Research: T-cells play a decisive roll in tumor defense. The recognition process of the T-cell in general and the recognition of tumor targets in particular are not understood at all. One clue to this understanding is the biochemical nature of the T-cell antigen recognition system.

Project Officer: Ms. Judith. M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $70,991

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator:	Dr. Chen K. Chai
Name/Address	Jackson Laboratory
Performing Organization:	Bar Harbor, ME

Contract Number: N01-CB-74181
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Elucidate the reasons that $\underline{A^y}$ mice are more susceptible to lung tumors than $\underline{aa}$ mice.

Approach: Study the carcinogenic responses of the homozygotes and heterozygotes for the different yellow and non-yellow alleles at the agouti locus.

Progress: It is of interest to note that, as a single gene model underlying tumor susceptibility, the yellow mice ($\underline{A^y a}$), a mutation at the agouti locus (A), are found to be more susceptible to lung tumors than the non-agouti ($\underline{aa}$) mice with the same genetic background. That a higher rate of somatic cell mutation is occurring in the yellow mice is hypothesized as an explanation. The agouti locus is a complex one, consisting of a large amount (or a series) of redundant nucleotide base sequences. The occurrence of mutation in the somatic cells contributes to the origin of tumor cells. This investigation is to test this hypothesis by comparing the lung tumor incidence between the homozygous yellow mice (except the lethal yellow) and the heterozygotes. A greater tumor incidence or number of tumors in the heterozygotes than in the homozygotes would support the present hypothesis. Mouse stocks carrying different yellow genes on a common genetic background are being produced. We have begun a course of urethane (a carcinogen) injection in these mice. In two months we will begin the autopsy and examination for the number of lung tumors and the percentage of mice developing tumors in each genotype.

Significance to Cancer Research: To determine the role that a gene, or a specific genetic locus, plays in cancer development. The results of this investigation would specifically concern the mutation theory of neoplasm.

Project Officer: Mrs. Judith M. Whalen
Program: Tumor Immunogology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $48,285

CONTRACT RESEARCH SUMMARY

Title: The Development and Maintenance of New Congenic Mouse Strains

Principal Investigator:	Dr. Edwin D. Murphy
Name/Address	Jackson Laboratory
Performing Organization:	Bar Harbor, ME

Contract Number: N01-CB-74174
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Develop and maintain new congenic mouse strains as models for determining basic mechanisms of lymphoproliferation and autoimmunity and their role in tumorigenesis.

Approach: Isolation of genetic factors controlling lymphoproliferation and autoimmunity and development of congenic models by selection and backcrossing for mutant gene lpr and establishing reciprocal F_1 hybrids for strain BXSB. Production of basic models for distribution.

Progress: The discovery of a mutant gene, lpr (lymphoproliferation), controlling massive T cell proliferation and autoimmunity opened the way for the development under this new contract of the first congenic inbred strains that permit fully controlled studies of the effects of spontaneous autoimmunity on tumorigenesis. The transfer of lpr to reference strain MRL-+/+ has now reached the 6th backcross generation (N6) establishing highly congenic inbred strain MRL-lpr/lpr with estimated residual heterozygosity <0.4%. During the period of development and breeding colony expansion, it has been possible to make available for preliminary study by other investigators limited numbers of the original models, strains MRL/l, MRL/n, and BXSB. Transfer of lpr to strains of major importance in cancer research is now at the backcross generation indicated in parentheses: A/HeJ (3), BALB/cJ (4), C3H/HeJ (3), C57BL/6J (6), C58/J (3), NZB/BlNJ (4), and SJL/J (6). A second model for lymphoproliferation and autoimmunity, recombinant inbred strain BXSB, shows inheritance as an autosomal dominant trait with acceleration of the disease in F_1 hybrid males produced by a factor contributed by the BXSB male parent.

Significance to Cancer Research: These cogenic models open the way to precisely controlled study at the molecular and cellular levels of the interaction of genes, viruses, and sex-related factors in autoimmunity and regulation of lymphocyte subset proliferation, and the role of autoimmunity and the immune processes in tumorigenesis.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $36,607

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator:	Dr. Michael Edidin
Name/Address	Johns Hopkins University
Performing Organization:	Baltimore, MD

Contract Number: N01-CB-43922
Starting Date: 5/1/74 Expiration Date: 4/30/78

Goal: Show differences in the diffusional mobility of surface proteins when comparing transformed and nontransformed cells. Factors affecting or changing diffusion rates, if elucidated, are to be used in modifying cell behavior and growth properties.

Approach: Measure lateral diffusion of membrane antigens in a wide range of cells using two techniques: (1) Formation of heterokaryons and analysis of the antigen distribution in the population; and (2) surface fluorescent labeling cells, then bleaching a spot in the label and measuring return of fluorescence to the spot.

Progress: A new laser photobleaching apparatus has been built and tested, allowing rapid comparison of lateral diffusion rates of various surface markers over numerous cell types, normal and transformed. A variety of Fab and paucivalent lectin reagents have been prepared for these studies. The cell fusion method of analysis of lateral diffusion in membranes has been used to further explore the effects of altered membrane potential on diffusion rates. It appears that calcium fluxes across the membrane, in response in potential changes, play a part in altering lateral diffusion rates. Photobleaching has also been used in a classical analogue of malignant systems--the mammalian embryo. Single cell techniques indicate that there are major changes in lateral diffusion of both membrane proteins and membrane lipids after activation of a previously quiescent, non-dividing cell, the egg by sperm (normal fertilization).

Significance to Cancer Research: Since membrane diffusion rates may be controlled by altering transmembrane potential, it may be that treated, transformed cells will revert to nontransformed in growth properties. Also, if the difference in diffusion rates between transformed and nontransformed cells holds consistently, it may be useful as a diagnostic aid.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Mechanisms for Cell-Mediated Destruction of Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: NO1-CB-43932
Starting Date: 5/1/74 Expiration Date: 4/30/78

Goal: This contract has been aimed at a better understanding of the mechanism by which human K cells destroy antibody coated tumor cells.

Approach: The cytolytic activity of human K cells has been quantitated by following the ^{51}Cr release from Chang cells coated with rabbit and human IgG antibody. The events leading to inhibition of K cell function by immune complexes have been investigated and experiments contrived to determine whether K cell function could be restored following such inhibition.

Progress: Studies on the inhibition of K cell function by a variety of IgG preparations revealed that the inhibitory capacity of IgG was determined by ligand (FC) multivalency. Unlike the binding of IgG to K cell Fc receptors, the IgG induced inhibition of K cell activity was found to involve a temperature dependent event. These and other findings led to the conclusion that IgG induced inhibition of K cell function resulted from the redistribution of Fc receptors on the K cell surface, leading to their eventual modulation. studies on the restoration of K cell function following inhibition with immune complexes revealed that inhibition could not be reversed by simply washing or culturing the inhibited K cells in standard culture medium. Restoration could be achieved if inhibited K cells were treated with a pronase solution (0.5%) and then cultured 37°C, >20 hrs). In this case, restoration could be attributed to the removal of cell bound immune complexes together with K cell Fc-receptors followed by the regeneration of new Fc receptors upon culture. Although not readily explicable, restoration could also be accomplished by simply culturing inhibited K cells in medium containing normal human serum.

Significance to or Cancer Research: These studies may permit a better understanding of the putative role of immune complexes in blocking cell mediated tumor cell destruction.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator:	Dr. Eva Klein
Name/Address	Karolinska Institute
Performing Organization:	Stockholm, Sweden

Contract Number: N01-CB-64023
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: Characterizing the naturally occurring cytotoxic cells (NK cells) in mice and man, partly to assess their significance and partly to develop methods to remove them, in order to reveal possibly specific reactions that are otherwise masked by them.

Approach: Lymphocyte fractionation and lymphocytotoxicity in human systems; the same in combination with _in vivo_ rejection tests in mouse systems.

Progress: The insensitivity of some highly NK-cell sensitive Moloney lymphomass to T-cell killing was shown to be an inherent membrane property of the target cells and not due to the absence of the potentially insensitive target site. A difference was found between the ability of in vivo maintained YAClymphoma and its in vitro subline to generate cytoxoxic T-sites in vitro, perhaps related to the ability of the in vivo YAC tumor to generate suppressor cells that counteracted the priming effect of syngeneic but not of allogenic tumors. A close parallellism was found between the hybrid resistance against bone marrow grafts and NK cell mediated resistance. NK-activity of radiochimeric animals corresponded to the bone marrow donor, not the recipient host. Similar findings were made on "age" chimeras. Murine and human NK cells had a certain cross reactivity, but with a better fit for targets of the corresponding species. Genetic patterns of resistance were identified in relation to the NK-mediated lysis and in vivo $f_1$hybrid resistance (presumably NK-mediated) against a variety of tumors.

Significance to Cancer Research: It is essential at this time to distinguish between nonspecific and specific killer effects in both mouse and human tumor systems and to initiate studies on the possible _in vivo_ relevance of _in vitro_ lymphocytotoxcitiy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $49,000

CONTRACT RESEARCH SUMMARY

Title: Role of the Macrophage in Tumor Resistance

Principal Investigator: Name/Address Performing Organization:	Dr. D. S. Nelson Kolling Institute of Medical Research Royal North Shore Hospital St. Leonards N.S.W., Australia

Contract Number: NO1-CB-63973
Starting Date: 7/16/75 Expiration Date: 10/31/78

Goal: To determine what roles macrophages may play in resistance to tumors and whether they can be exploited in immunotherapeutic strategies.

Approach: The growth of grafts of chemically induced tumors is measured in the feet of mice. Studies are made of the effects of various agents and procedures on the growth rate of tumors in normal and immune mice. The mice are immune either (a) during carriage of a tumor (concomitant immunity, at first specific and later nonspecific) or (b) after injection of a small number of tumor cells (sinecomitant immunity, always specific). In parallel, the effects of macrophages and other cells on the survival and multiplication of cultured tumor cells are measured.

Progress: Tumor rejection could be initiated by T cells with Ly markers (1^+, 2^-, 3^-) characteristic of T cells initiating DTH reactions. Intraperitoneal (i.p.) challenge of mice with the tumor to which they were immune specifically triggered influx into the peritoneal cavity of cells with the morphology of macrophages. Further investigation of anti-tumor effector cells has shown that they occur not only in the peritoneal cavities of tumor-immune mice, but also in peritoneal exudates induced in normal mice by proteose peptone, or following infection with Listeria, Salmonella or BCG. The cells were separated on the basis of sedimentation velocity. Large macrophages had an entirely nonspecific cytostatic effect. Smaller moncytoid cells had a tumor-selective, but not individual tumor-specific, slow cytotoxic effect, different from the effect of NK cells on susceptible targets. Specific i.p. challenge of tumor immune mice led to a selective increase in cells of the latter type.

Significance to Cancer Research: Immunity to the tumors studied involves increased production of naturally occurring nonspecific effector cells (macrophages) and the development of a specific delivery mechanism, akin to elayed-type hypersensitivity, which overcomes the antagonistic effects of tumor cell products.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $57,109

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Bone Marrow Transplantation

Principal Investigator:	Dr. Ross S. Basch
Name/Address	New York University Medical Center
Performing Organization:	New York, NY

Contract Number: N01-CB-74176
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To develop an animal model for transplantation of selected bone marrow cells of defined function and developmental potential.

Approach: To study the relationship between the hematopoietic precursors of immunocompetent T cells and the "natural killer cells" of bone marrow and spleen in mice.

Progress: We have begun to explore the relationship of NK (natural killer) cell lineage. Initial studies indicated that antiserum which is routinely used as an anti Ly 1.2 reagent contains antibodies against thymocyte precursors. These antibodies are unrelated to the α Ly activity of the serum. The serum has been reported by Glimcher et al. to have α NK activity, but we have had great difficulty in confirming this. We have shown that NK cells bear on their surface an antigen (tentatively designated as SC-1) which is also found on CFU-S, thymocyte precursors and some B cell tumors. Using a negative immunoselection technique, we have obtained cloned cell lines of a murine lymphoma (RLO 1) which appear to differ from each other only by the presence of the SC-1 antigen. These cell lines are now being used to prepare antisera specific for the SC-1 antigen. The presence of SC-1 on NK cells suggests that these cells are relatively immature but does not establish their relationship to prothymocytes.

Significance to Cancer Research: If natural resistance to malignancy is mediated by an immature member of the T cell lineage, it may be possible to influence the rate of passage of cells through this compartment and alternately influence the magnitude of natural response.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $71,776

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Name/Address Performing Organization:	Dr. Gerald L. Bartlett Pennsylvania State University Milton S. Hershey Medical Center Hershey, PA

Contract Number: N01-CB-64039
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To test whether "spontaneous" murine tumors appear to be non-antigenic due to processes which are known to interfere with cell-mediated cytotoxicity.

Approach: Obtain new tumors likely to be nonimmunogenic ("spontaneous" or induced by means not associated with strong tumor antigens). Test immunogenicity of the tumors in sensitive in vivo tests (immunization-challenge, Winn Assay). Determine the effect on immunogenicity of procedures intended to: 1) deplete suppressor activity; 2) remove blocking factors; or 3) "reactivate" sensitized cells.

Progress: Several new "spontaneous" tumors were established, and treatments were initiated to obtain several classes of induced tumors: film-induced sarcomas, urethan-induced lung adenomas, sarcomas derived from spontaneous transformation of cells in diffusion chambers, endogenous hormone-induced tumors. Immunogenicity teste were begun on two spontaneous diffusion chamber tumors already available. Several additional spontaneous tumors were established: sarcomas, lymphomas, leukemias, hepatomas, and mammary carcinomas. Some induced tumors were established. One spontaneous tumor (squamous cell carcinoma of skin) was shown to be antigenic. Several hepatomas grew poorly after s.c. inoculation, but metastasized from the site to the liver. One sarcoma, which was nonimmunogenic in standard tests, was weakly immunogenic after he syngeneic hosts were treated in a way previously shown to deplete suppressor cells.

Significance to Cancer Research: Apparently, the immunity that can be detected in vitro is ineffectual for tumor rejection in vivo. This project is to to examine the role of several processes which might be interfering with tumor rejection immunity. Such information would guide subsequent efforts to improve methods for immunotherapy of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $66,610

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Name/Address Performing Organization:	Dr. R. L. Marquet Radiobiological Institute Rijswijk (KH), The Netherlands

Contract Number: N01-CB-64038
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: Investigations on the immunogenicity of tumors in syngeneic rats. The intent is to survey a large variety of tumors that share the following characteristics: (1) arise spontaneously in low frequency and (2) arise following exposure to various dosages of irradiation.

Approach: The ultimate tests of immunogenicity will be in vivo challenge-protection experiments in syngeneic rats, using early transplant generations of the tumors.

Progress: Of sixty transplantable rat tumors harvested, thirty-one passed the challenge protection experiments and for ten tumors TD-50's (approach II) were determined in control and immunized animals. Using challenge protection seven "immunogenic" tumors have been identified and three were checked for immunogenicity by approach II. For two tumors the original immunogenicity was confirmed in approach II.

Significance to Cancer Research: Several laboratories have reported that "spontaneous" animal tumors have little or no immunogenicity as measured by challenge-protection experiments. If this observation is generally correct, it would have important implications for ideas about immunotherapy and immunodiagnosis.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $2,084

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. W. Hallowell Churchill
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-64036
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To determine if sensitized lymphocytes stimulated with tumor antigen generate macrophage-activating factor (MAF) or factors capable of enhancing macrophage-mediated cytotoxicity. To further define the nature of the interaction between MAF and macrophages. To explore the mechanisms by which macrophages, once activated, kill tumor cells.

Approach: Macrophages are activated in vitro by treatment in suspension culture with MAF-rich supernatants. Their cytotoxicity for tumor cells labeled with ^{3}H-proline or ^{3}H-thymidine is then determined.

Progress: We have shown that partially purified peritoneal lymphocytes from tumor immune animals produce macrophage activating factor (MAF) when stimulated by the immunizing tumor. Further investigation of the role of sugars in the interaction between MAF and macrophages has shown that fucose consistently inhibits the effect of MAF on macrophage cytotoxicity, whereas other sugars such as rhamnose and alpha-methylmannoside do not. Furthermore, removal of cell surface fucose by treating macrophages with fucosidase also inhibits the induction of cytotoxic macrophages by MAF. Cell surface sialic acid may also participate in the MAF macrophage interaction, since treatment of macrophages with neuraminidase renders them refractory to the effects of MAF. Finally, major progress has been made in analyzing the mechanisms by which macrophages, once activated, kill tumor cells. Experiments with inhibitors of protein synthesis show that inhibition of cytotoxic effect roughly parallels inhibition of protein synthesis. Furthermore, binding of tumor cells to activated macrophages is also decreased in the dose dependent fashion by these inhibitors. Results with inhibitors of microtubules and of microfilaments are similar.

Significance to Cancer Research: Macrophages are an important component of the host response to tumor antigens. Our studies are designed to elucidate the mechanisms by which macrophages acquire enhanced cytotoxicity for tumor cells and the mechanisms by which activated macrophages kill tumor cells.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $74,790

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune-Related Cell Lines

Principal Investigator: Name/Address Performing Organization:	Dr. Melvin Cohn The Salk Institute San Diego, CA

Contract Number: N01-CB-23886
Starting Date: 2/26/74 Expiration Date: 2/25/79

Goal: To supply the scientific community with immune-related lines important in the study of tumor immunology and to increase the library of useful lines as they are characterized in our laboratory.

Approach: Since this is essentially a contract to supply cell lines, there is little experimental work involved. However, the general thrust of the laboratory involved the isolation of immune-related lines of the thymus and bone marrow-derived lineages by the use of leukemia viruses, cell fusions, and chemical carcinogens. As new lines become available, they are introduced into the contract.

Progress: Normal immune-related lines are usually dead-end and do not grow in culture. It is necessary, therefore, to transform them in order to isolate and lone them *in vitro*. The contractor has a vast experience with the induction of myelomas, lymphomas, macrophage tumors, and other immune-related cells using chemical carcinogens and leukemia viruses. This work has formed the basis for the present contract to supply cell lines. During the present contract year, these studies were extended to isolation of normal cells using cell fusion techniques. Antibody-producing cells as well as T cells of the cytotoxic and cooperating lineages were isolated.

Significance to Cancer Research: The lines in this contract are used by hundreds of laboratories for studies on tumor-specific antigens, antibody structure, immune-related cell functions, somatic genetics, and cell fusions. All of these subjects are major categories under the NCI Cancer Research Program, and the cell lines from this laboratory are key tools in these investigations.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $74,660

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Dr. Stuart Schlossman
Name/Address Sidney Farber Cancer Center
Performing Organization: Boston, MA

Contract Number: N01-CB-53881
Starting Date: 5/1/75 Expiration Date: 4/30/78

Goal: The overall goal of this proposal was to investigate the sensitization of human lymphocytes to syngeneic and autologous and solid tumor cells in order to generate specific cytotoxic cells utilizing lymphocytes from HLA-MLC-matched siblings with the view of improving our understanding of the subpopulations of cells involved and the mechanisims by which specific tumor cell immunity is generated.

Approach: These studies were facilitated by the development of techniques capable of isolating lymphocyte subpopulations, in vitro sensitization to alloantigens, and the development of specific cytotoxic cells directed at panels of chromium labeled targets of known HLA type on both normal allogeneic and tumor cells.

Progress: The data collected by the contractor indicates that leukemic blasts can trigger MHC-identical lymphocytes to differentiate into cytotoxic cells reactive to leukemic blasts but not to normal MCH-identical PHA blasts, provided a third party helper stimulus is present. Further, it was shown that two subsets of cells --$TH2^-$ and $TH2^+$--are required to generate this cytotoxic reaction and that the specific subset of T cells which kills are $TH2^+$. The contractor has also demonstrated that the cytotoxicity of $TH2^+$ and $TH2^-$ cells, when recombined, was markedly greater than either subset alone. These studies also indicate that $TH2^-$ cells function primarily as a helper cell population in collaboration with $TH2^+$ cells in the generation of cytotoxic $TH2^+$ cells. More importantly, these studies provide insight into the regulatory cellular events necessary to generate an immune response to tumor cells. Removal of suppressor cells or addition of helper cells facilitates sensitization to tumor cells in man.

Significance to Cancer Research: The precise dissection of the cellular controls of the cytotoxic response to one's own tumor and the demonstration that subsets of cells can both enhance and suppress this response is critical for our understanding of the mechanisms of tumor cell growth.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator: Name/Address Performing Organization:	Dr. Yashar Hirshaut Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: NO1-CB-53942
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To demonstrate new tumor-related antigens associated with human sarcomas. Antigens found, as well as those already demonstrated, are to be characterized to determine their nature, specificity, and distribution.

Approach: The serologic responses of patients with sarcoma to their tumor cells are assayed by immunofluorescence, complement fixation, and microcytotoxicity tests. Preliminary characterization of antigens is achieved by immunologic means. Efforts are under way to isolate, purify, and biochemically analyze specific antigens.

Progress: The conditions for growing human sarcomas in culture and maintaining antigen production have been established. Of particular interest has been the requirement for human cord serum as the source of complement in the S_3 assay. Guinea pig complement is usually inactive or gives very low titer reactions. Collaborative studies have revealed that there are quantitative differences in the components present in the complements of different species, but consumption studies so far show no differences in their attachment to the sarcoma antigen-antibody complexes. S_1 and S_3 are now clearly established as heterophile antigens unrelated to those associated with sheep red blood cells such as the Forssman, infectious mononumleosis, and serum sickness antigens. Antibody to serum sickness antigen has, however, also been found to be more prevalent in patients with malignancy. Leukemic blasts have heterophile antigens present on their surface which are not found on more mature cells. Such antigens are also associated with oncorna virus isolates. Efforts at biochemical purification of S_3 are underway and indicate that it may be structurally distinct from the cross-reacting antigen present in guinea pig kidney cells.

Significance to Cancer Research: Definition and characterization of human tumor antigens should be of value in permitting early diagnosis and the further development of immunotherapy for cancer. Such antigens may also provide new insight into changes that accompany malignant transformation.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical 78 Group: Committee on Cancer Immunobiology
FY 1978 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Name/Address Performing Organization	Dr. Fung-Win Shen Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-64031

Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To develop a system for measuring the immune capability of T cells against syngeneic chemically-induced tumors of C57BL/6 mice in vivo. By use of this system to delineate the contribution of tumor-specific T cells to immunity against tumors in vivo. To determine which functionally distinct T-cell populations may be responsible for abrogating this destruction in vivo. To determine whether any cross-reactions of different chemically-induced tumors can be demonstrated by cellular as well as serological methods.

Approach: The in vivo assay will be applied to a series of chemically-induced C57BL/6 transplanted tumors to establish the role of the Lyt-1, Lyt-23, and Lyt-123 functional subclasses, alone and in combination, in the immune response to chemically-induced tumors in vivo.

Progress: Further evidence that the Lyt-123 set is necessary for rejection, under the conditions of the Winn assay, has come from experiments showing that neither the Lyt-1 set alone nor the Lyt-23 set alone, nor any combination of these two sets, in feasible numbers, will completely supress Meth 4 sarcoma in syngeneic hosts. We do not infer that Lyt-123 is the effector-killer cell in this system, but rather that Lyt-1 amplifiers and Lyt-23 killer-effectors are drawn from a specifically-primed Lyt-123 pool. The grounds for this conclusion are Stutman's work on cytotoxicity against sygeneic mammary tumor cells in vitro, in which the Lyt-123 set is required for maximal cytotoxicity in a long-term assay, despite the fact that the killer-effectors at all phases of cytotoxicity have the phenotype Lyt-23.

Significance to Cancer Research: A foreseeable outcome of this study is that elimination of one functionally distinct Lyt cell population may augment tumor rejection, and another suppress it. At this point it would not be difficult to envisage research programs directed to the application of such methods to therapy of at least some human cancers.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $82,170

CONTRACT RESEARCH SUMMARY

Title: Automated Systems for HLA Typing

Principal Investigator:	Dr. F. Carl Grumet
Name/Address	Stanford University
Performing Organization:	Stanford, CA

Contract Number: N01-CB-53935
Starting Date: 6/16/75 Expiration Date: 1/31/78

Goal: To complete the final phase of development of an automated HLA typing system and to demonstrate the effectiveness of this system.

Approach: Typing trays are washed and spun. Total fluorescence (using fluorescein diacetate as a marker of cell viability) in each well is proportional to total cell viability, so that test readings are converted from an enumerative process to a machine-readable quantitative process.

Progress: The automated system under study was based on a modification of a standard lymphocyte microcytotoxicity assay. The automated stage was refined to read a 60 or a 72-well test plate in 16 seconds, and a multiple carrier for washing test plates was developed. Minicomputer analysis programs for tails of reactions were implemented, reducing data analysis time by 90%. Overall efficiency of the typing system was not completed because of manufacturer quality control problems with the 72-well test plates. Final demonstration of efficiency of the completed system will be performed. Preliminary experiments have shown that mixed lymphocyte culture reactions may be quantitated by the automated scope. In addition to the possibility of using the automated scope for MLC typings, preliminary experiments with B lymphocyte typing suggest that after further development, the automated scope may also be suitable for testing for HLA-DR antigens.

Significance to Cancer Research: Animal models demonstrate that genes linked to the species MHS can affect susceptibility to different viral-induced tumors. Improved ability to perform HLA typing should permit fine genetic mapping of the human MHS and possibly help localize and identify any human disease susceptibility or immune response genes in that MHS region.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies of In Vitro Induction of Functional Differentiation of T Cells in Systems Relevant to Tumor Immunology

Principal Investigator:	Dr. Samuel D. Waksal
Name/Address	Tufts University School of Medicine
Performing Organization	Boston, MA

Contract Number: N01-CB-74150
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Study the role of thymus epithelium in the differentiation and transformation of lymphocytes, including isolation and characterization of prothymocytes from normal, athymic, and tumor-bearing mice.

Approach: Presentation of tumor and virus antigens to prothymocytes during in vitro differentiation to produce populations of antitumor effector cells. Analysis of the ability of thymus epithelium to induce T-lymphocyte differentiation in high leukemic strains (AKR/J and HRS/J) as opposed to control strains.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 6 Approach 4)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $85,618

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Marrow Transplantation

Principal Investigator:	Dr. Paavo Toivanen
Name/Address	Turku University
Performing Organization:	Turku, Finland

Contract Number: N01-CB-74177
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Study the origin and early differentiation of lymphoid stem cells in chickens.

Approach: Transplantation of stem cells or allogeneically educated, more mature cells into immunodeficient chicks or chicken embryos. Study the fate and function of transplanted cells by morphological and functional means. Determine whether stem cells originate in the yolk-sac or intra-embryonically.

Progress: During the present contract year, we have transplanted embryonic bone marrow cells of different stages of development into 15-day-old irradiated chicken embryos. To study the fate and differentiation of the transplanted cells, sex chromosome and Ig-allotypic markers have been utilized. To study stem cell maturation in allogeneic environment, serial transplantation of bursa cells in allogeneic and synegenic hosts have been used. Possibility for a bursal origin of T lymphocytes has been studied by transplanting chromosally marked bursa cells into immunodeficient chicken embryos. All of these experiments are still in progress. As most important of our findings, we consider the evidence obtained indicating that lymphoid stem cells are primarily derived rather from an intraembryonic source than from the yolk sac. This evidence is based on the use of yolk sac-embryo chimeras with sex chromosomes and IgG allotypes as markers.

Significance to Cancer Research: In order to understand disturbances in the immune surveillance system facilitating the development of malignancies, and to be able to manipulate the system therapeutically, detailed knowledge of its normal development is of fundamental importance.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $46,980

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-Binding T-Cell Receptors

Principal Investigator:	Dr. Joel W. Goodman
Name/Address	University of California
Performing Organization:	San Francisco, CA

Contract Number: N01-CB-74178
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: Purify T cell-derived antigen-binding receptors; evaluate new procedures for detection of antigen-binding T cells.

Approach: Use the response of A/J mice to chemically defined antigen (p-azobenzenearsonate) for these studies.

Progress: A/J mice make antibody to the azobenzenearsonate (ABA) determinant, which carries a major idiotypic specificity (CRI). T cell-mediated ABA-specific responses are also made which can be assayed as help or suppression for anti-hapten Ab responses. Immune animals develop T cells which bind ABA; a variable proportion of ABA-binding T cells from individual mice can be inhibited by anti-CRI, indicating that their receptors and conventional anti-ABA Abs share a similar or identical idiotypic marker. We now find that mice immunized with ABA-mouse IgG develop suppression for anti-DNP responses to doubly conjugated ABA, DNP-proteins. This suppression is specific for ABA and mediated by T cells in cell transfer experiments. ABA-binding T cells from suppressed animals have been purified by removing B cells on plastic dishes coated with anti-mouse Ig Ab, followed by binding the non-adherent cells to dishes coated with ABA-protein. Of the cells recovered by elution from the ABA plates, 70-90% bind ABA immediately afterward. Up to 50% express the CRI in binding assays. We now plan to hybridize these cells with lymphoma cell lines to get stable hybrids which express the CRI.

Significance to Cancer Research: (NCP Objective 6 Approach 4)
Objective 3 Approach 4)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $76,435

CONTRACT RESEARCH SUMMARY

Title: Cell Surface Immunobiology of Metastatic Tumors

Principal Investigator: Name/Address Performing Organization:	Dr. Garth L. Nicolson University of California Irvine, CA

Contract Number: N01-CB-74153
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study surface properties of tumor cells important in malignant tumor spread.

Approach: Use or derive rat and/or mouse variant cell lines with altered *in vivo* properties such as the ability to metastasize via lymphatics or blood to specific secondary sites.

Progress: Immunobiology of lymphocyte-resistant and sensitive metastatic melanoma. We are determining the surface properties important in the escape of metastatic melanoma cells from lymphocyte-mediated cytotoxicity, the role of cell-mediated immune response in blood-borne melanoma metastasis and the possibility that stimulatory lymphokines are released by lymphocytes curing interactions with metastatic tumor cells. Development and characterization of metastatic rat mammary adenocarcinoma cell lines. Using carcinoma line 13762NF, we are cloning and selecting variant cell lines with differing *in vivo* metastatic properties. These lines metastasize from s.c. sites to regional lymph nodes and then spread to lungs via a blood-borne route. Cell surface changes on highly metastatic 13762NF lines and their clones are being investigated by immunological, biochemical, and morphological means to determine surface characteristics important in mammary tumor metastasis. Development and characterization of metastatic neuroblastoma lines: Neuroblastoma lines metastatic to liver are being selected from cell lines of low *in vivo* malignant potential to determine host and tumor properties important in neuroblastoma metastasis.

Significance to Cancer Research: A leading cause of death due to cancer is tumor metastasis, but little is known concerning the tumor and host properties important in metastatic tumor spread. Detailed information on the life threatening, highly metastatic cell variants that exist in the primary tumor at low frequencies is necessary to develop suitable detection and therapeutic procedures to prevent metastasis.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $95,783

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator:	Dr. Eli E. Sercarz
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-43972
Starting Date: 6/1/74 Expiration Date: 12/31/77

Goal: To understand the role of the various genes involved in the regulation of the T and B cell responses to a well-characterized group of protein antigens, the lysozymes; and, hopefully, to understand the submolecular basis for discrimination between non-immunogenic and immunogenic lysozymes.

Approach: Using chicken egg white lysozyme (HEL), its reduced carboxymethylated derivative (RCM-HEL), its congeners from other gallnaceous egg whites, peptide derivatives, and human lysozyme (HUL), we have studied recognition at the T cell and B cell levels. T cell reactivity has been judged by induction of T cell proliferation, T cell rosetting, and functional effects: studies on specificity of suppression and help. B cell responses were studied by isolectric focusing of serum antibody and by plaque-forming cell analysis.

Progress: Mice of the $H\text{-}2^b$ haplotype show immune unresponsiveness to HEL being under control of two recessive genes within H-2, one in the K-1A region and a second to the right of 1-B. Evidence for only a single recessive gene, in the K-1A region, was found with HUL. Two mutants of $H\text{-}2^b$ which appear to be point mutants in the K protein of the HEL complex respond to HEL but not to HUL. Studies with recombinant inbred strains give evidence for at least one gene outside H-2 which can allow response to HEL in $H\text{-}2^b$ mice. With HUL, it appears that two non-H-2 genes, acting in concert can overcome $H\text{-}2^b$ nonresponsiveness. HEL and RCM-HEL are completely crossreactive at the T cell level. Neither molecule induces T cell proliferation in $H\text{-}2^b$ mice. However, a large peptide prepared by removing two small peptides from the N-terminal and C-terminal of RCM-HEL gives good stimulation of T cell proliferation. This result suggests that a key suppressor determinant has been amputated from RCM-HEL. Direct evidence for suppressor T cells has been obtained.

Significance to Cancer Research: These results suggest through proper chemical alteration of protein molecules that it is possible to manipulate the immune response.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen Binding T-Cell Receptors

Principal Investigator: Name/Address Performing Organization:	Dr. Frank W. Fitch University of Chicago Chicago, IL

Contract Number: N01-CB-74149
Starting Date: 9/19/77 Expiration Date: 9/18/78

Goal: Characterize antigen receptors on rat T cells which are specific for rat alloantigens.

Approach: Preparation of monospecific anti-idiotypic antisera; development of continuous cell lines of rat lymphoid cells which secrete homogeneous antibody reactive with rat alloantigens; screening of various rat T-cell populations for the presence of relevant idiotypic receptors.

Progress: During the first six months of this contract, several important objectives have been attained; 1) two mutant sublines of rat myeloma cells suitable for somatic cell hybridization have been derived; 2) a simplified and efficient method of fusing lymphocytes to mutant myeloma cells has been developed; 3) six continuous hybridoma cell lines secreting cytotoxic antibody against Brown Norway alloantigens have been derived, cloned, and partially characterized; 4) seven continuous hybridoma cell lines secreting noncytotoxic antibody against Brown Norway alloantigens have been derived and cloned; 5) methods for biosynthetically labelling hybridoma antibodies have been developed; 6) a radioimmunoassay for detection of anti-idiotypic antibodies in this system has been developed; 7) anti-idiotypic antibodies have been prepared against several of the hybridoma antibodies, and 8) the biological effects *in vivo* and *in vitro* of the hybridoma antibodies are being evaluated.

Significance to Cancer Research: (NCP Objective 6 Approach 4)
Objective 3 Approach 4)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $78,325

CONTRACT RESEARCH SUMMARY

Title: Studies by Which Tumors Avoid Destruction by the Immune System

Principal Investigator:	Dr. Eugene M. Lance
Name/Address	University of Hawaii
Performing Organization:	Honolulu, HI

Contract Number: N01-CB-63978
Starting Date: 8/16/75 Expiration Date: 8/15/78

Goal: This project investigates the phenomenon wherein animals bearing tumors lose the ability to recruit lymphocytes in response to an immunogenic challenge and, as a consequence, develop a generalized and nonspecific depression of their immunological capacity.

Approach: Cell-free fluids are obtained from tumor-bearing animals and inoculated into tumor-free recipients. These recipients are studied in assays which measure independently the ability to recruit or trap lymphocytes. The immunological performance of such recipients in response to conventional antigens, skin allografts, and tumor challenge are measured as well.

Progress: The transfer of cell-free fluid from tumor-bearing animals (ATF) has been found to interfere with lymphocyte recruitment in otherwise normal recipients. This diminution of trapping is nonspecific in the sense that the effect is independent of the challenging immunogen. ATF-treated animals have a reduction in immune responsiveness, both humoral and cellular. Further, the antibody titers to conventional antigens are reduced. The possibility that these effects may be due to a passenger virus present within the tumor lines was considered and has been confirmed. Lactic dehydrogenase virus was found to exist as a passenger in all laboratory tumors studied. When tumor lines prepared free of LDV, the effects mentioned above were no longer observed.

Significance to Cancer Research: The failure of lymphocyte recruitment and the ensuing generalized immunodepression seen in tumor-bearing animals could interfere with the host's attempts at tumor rejection and also render such hosts less able to ward off infections. That many of the so-called immunological effects commonly studied in the laboratory analysis of tumor immunity are attributable to contaminating virus seems almost certain and human tumors may harbor viruses which produce similar effects.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Name/Address Performing Organization:	Dr. Pekka J. Hayry University of Helsinki Helsinki, Finland

Contract Number: N01-CB-64032
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To analyze the origin, type, and receptor specificity of allograft-infiltrating cells, as well as their functions in the rejection process and later to perform similar studies with syngeneic tumor grafts.

Approach: The following approaches have been used: 1. Mouse sponge matrix allografts (SMG); 2. Human and rat kidney allografts; 3. Human cancers. In SMG the infiltrating cells were recovered by compression, the rest of the grafts were disaggregated by collagenase-DNAse, and the infiltrating cells were separated from tumor or parenchymal cells by 1-g velocity sedimentation.

Progress: The SMG is first infiltrated by T killer cells, later also cytotoxic non-T lymphocytes and monocytes participate in the infiltrate. Most of the infiltrating cells, including cells of the monocytic lineage, carry idiotypic receptors to the graft donor antigens. A mixed population of cells, including (in this order of magnitude) monocytes/macrophages, T lymphocytes, non-T lymphocytes, B cells, plasmablasts/plasma cells, and T lymphoblasts infiltrate rat and human kidney allografts. The infiltrating cells are far more cytotoxic than blood or (in rat) spleen lymphocytes to donor-derived lymphoid target cells. Variable inflammatory population consisting of (in this order of magnitude) IgG-FcR positive monocytes/macrophages, null lymphocytes, T and B lymphocytes but no or very few blast- and plasma cells infiltrate human cancers. Even when blood leukocytes are cytotoxic to the patient's own cancer cells, the infiltrating cells are not. The sequence of appearance of the different types of killer cells in allografts, and the reason(s) for the lack of cytotoxicity within cancers are under analysis.

Significance to Cancer Research: Elucidates in the situ cytotoxicity mechanisms in the anti-allograft and anti-cancer immune response.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $53,650

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T Cells

Principal Investigator:	V. E. Olavi-Makela
Name/Address	University of Helsinki
Performing Organization:	Helsinki, Finland

Contract Number: N01-CB-43923
Starting Date: 6/1/74 Expiration Date: 5/31/78

Goal: To find out whether the receptor antibodies that mediate recognition by T lymphocytes are controlled by the Mendelian genes that control specificity of circulating antibody molecules (V_H genes).

Approach: To determine whether T lymphocytes of an individual carry the V_H gene controlled idiotypes that are carried by Ig-molocules of this individual.

Progress: Before the present contract year, we had found a Mendelian polymorphism in mouse antibodies against three haptens (NP, ABA-HOP and NBrP). For example, anti-NP antibodies of C57BL mice have systematically different V_H regions than BALB antibodies against this hapten. We had prepared anti-idiotypic reagents against the products of the V_H NP gene of C57BL and against the V_HABA-HOP gene of A/J. In collaborative work with Rajewsky, we had found that nylon-adherant T cells have antibodies carrying the idiotypic and fine-specificity characteristics determined by the (Ig) genotype of the mouse. During the report period, we have found two new V_H genes; in antibodies to cloxacillin and oxazolone. We have prepared anti-idiotypic reagents against the anti-oxazolone antibody of BALB/c. We have demonstrated a hapten-specific T-lymphocyte help with two of these haptens, --NP and oxazolone. The next step was to demonstrate that idiotypic antibody on the T cells in functionally relevant: lethally irradiated mice received BSA-immunized B cells and NP-immune or non-immune T cells (nylon wool) plus NP-BSA challenge. The former group developed four times as many BSA PEC as the latter, and the increase was inhibited. Recipients received 0.1 ml of mouse anti-idiotype serum.

Significance to Cancer Research: T cells play an important role in the body defenses against cancer. Elucidation of their mode of recognition is important.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $28,800

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Geoffrey Haughton University of North Carolina Chapel Hill, NC

Contract Number: N01-CB-74182
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Investigate the finding that a polymorphic gene or genes, linked to the H-2 complex in mice, determines susceptibility to primary sarcoma induction following neonatal injection of RSV-containing chicken tumor material.

Approach: Investigate the mechanism of action of the genes to determine whether differences in immune response to tumor antigens are involved. Explore the finding that both dominant and recessive resistance exist. Test for immune surveillance and immune stimulation.

Progress: We have shown that genes regulating susceptibility or resistance segregate with the H-2 complex. We cannot find evidence for any other influential gene. Resistance and susceptibility are condominant; heterozygotes display an intermediate level of susceptibility. Strains which are relatively resistant to primary tumor induction (H-2^{d}, H-2^{s}) mount a more vigorous tumor neutralizing response, when immunized against the Rous tumor transplantation antigen, than do the relatively susceptible strains (H-2^{a}, H-2^{k}). Transfer of immune lymphoid cells to genetically identical baby mice, early in the latent period of tumor induction does not reduce tumor incidence to zero. Preliminary results indicate that differences in type of lymphoid cell transferred and in timing of administration may lead to either increase (immunostimulation) or decrease (immunosurveillance) in tumor incidence.

Significance to Cancer Research: We have demonstrated a clear link between genetic control of tumor susceptibility and genetic control of tumor specific immune responsiveness. Preliminary data suggest that different components of the immune response stimulate the growth of and eliminate tumors.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $76,118

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator:	Dr. Michael J. Embleton
Name/Address	University of Nottingham
Performing Organization:	Nottingham, England

Contract Number: N01-CB-64009
Starting Date: 6/18/76 Expiration Date: 6/17/78

Goal: To determine (a) the frequency of expression of tumor-associated antigens capable of inducing host resistance in spontaneously arising tumors of an inbred rat strain, and (b) the influence of these antigens on tumor development.

Approach: Spontaneous rat tumors arising in a normal breeding population are transplanted into syngeneic hosts and their immunogenicity is assessed by immunization/challenge protection studies in vivo, carried out mainly during early transplant generations. The transplantation studies are supported by serological testing for antibodies reactive against tumor cell surface antigens.

Progress: More than 100 spontaneous tumors have been transplanted into sygeneic rats over a number of years, and immunogenicity tests have been completed with 24 of them. Rats were immunized by surgical removal of a growing tumor transplant or by treatment with attenuated tumor, followed by challenge with viable tumor cells. Immunity to challenge with cell doses growing in control rats was induced against 7 of the 24 tumors. In most cases the response was weak, but in some cases immunity approaching the levels experienced with chemically-induced or virus-induced tumors was observed, this often being accompanied by a humoral antibody response to the tumor. In all cases the responses detected were specific for individual tumors. The adjuvant agents BCG and Corynebacterium parvum were tested for their ability to increase resistance to spontaneous tumors, but they were effective only in conjunction with immunogenic tumors.

Significance to Cancer Research: Spontaneous tumors are more relevant to human tumors than are experimentally-induced tumors. These studies therefore suggest that some human tumors could have specific antigens relevant for host immunity.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $61,595

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator:	Dr. Hans Wigzell
Name/Address	Uppsala University
Performing Organization:	Uppsala, Sweden

Contract Number: N01-CB-64033
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To define the physiology and characteristics of natural killer cells and other cells in the reaction against syngeneic tumor cells.

Approach: Combination of specific fractionation procedures does allow the select purification of certain subgroups of cells. Such subgroups will be analyzed as to specific tumor immune reactivity, in _in vitro_ or _in vivo_ systems using cells in normal or immune individuals.

Progress: "Natural killer" cells in the mouse as well as in man represent a new and interesting cell type. It is endowed with the ability to rapidly lyse, especially tumor target cells, _in vitro_ via a contactual mechanism. It was previously shown that this cell type is a thymus-independent cell, originating in the bone marrow at a certain age of the individual and quite susceptible to radioactive strontium. Chimera work has allowed the establishment of high and low NK animals by taking advantage of the genetic regulation at the stem cell level of NK generation. A highly positive correlation could be shown to exist between the NK levels in such T-deficient chimeras and their _in vivo_ resistance to transplantation of syngeneic lymphomas. These data suggest that natural killer cells are a most decisive cell group with regard to tumor resistance _in vivo_. In line with these findings are the more recent results that interferon and intereferon inducers including _Corynebacterium parvum_ seem to be major activating agents for NK cells _in vivo_. As such, agents are among the most promising for "immune therapy" of tumors. This is in line with the above concept.

Significance to Cancer Research: Deeper understanding of relevant cell types involved in _in vivo_ resistance to syngeneic tumor cells is necessary before a predictable manipulation of the immune reactions against tumors will be possible.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Granulocyte and Macrophage Proliferation Control in Leukemia and Related Neoplasms

Principal Investigator:	Dr. Donald Metcalf
Name/Address	Walter and Eliza Hall Institute
Performing Organization:	Victoria, Australia

Contract Number: NO1-CB-33854
Starting Date: 6/20/73 Expiration Date: 10/19/77

Goal: To chemically characterize and purify the glycoproteins colony-stimulating factor (GM-CSF) controlling granulocyte and macrophage proliferation, analyze its mechanisms of action, and determine whether leukemic cells respond to this regulator.

Approach: The proliferation of normal or leukemic mouse or human granulocytes and macrophages was analyzed using an agar cloning technique, colony formation being dependent on the concentration of GM-CSF added. Three major source materials of GM-CSF were used: mouse lung conditioned medium, mouse spleen cell conditioned medium, and human placental conditioned medium.

Progress: Leukemic cells from patients with acute or chronic myeloid leukemia could be reliably cloned in agar, the growth pattern being a useful practical method of assistance in the initial diagnosis of difficult patients and in monitoring the response of patients to therapy. Initial growth patterns of leukemic cells were also a useful prognostic index of probable response to chemotherapy. In all of the 500 leukemic patients surveyed, at all stages of their disease, the leukemic population was totally dependent on the regulator GM-CSF for proliferation *in vitro*, a unique situation in tumor biology that offers alternative methods for therapy in these leukemias. Procedures were developed for mass-producing stable GM-CSF preparations capable of maximally stimulating human GM-colony proliferation. Procedures were developed that, for the first time, allowed the clonal proliferation in agar of mouse eosinophils, megakaryocytes and B-lymphocytes.

Significance to Cancer Research: The work has provided monitor systems now being used in many hospitals in the diagnosis and management of patients with myeloid leukemia and related disorders and in monitoring patients with marrow transplantation.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Bone Marrow Transplantation

Principal Investigator: Name/Address Performing Organization:	Dr. Donald Metcalf Walter and Eliza Hall Institute Parkville, Australia

Contract Number: N01-CB-74148
Starting Date: 9/20/77 Expiration Date: 9/19/78

Goal: Develop granulocyte-macrophage and lymphocyte progenitors from transplanted marrow cells.

Approach: Agar cultures were used to monitor precursor populations of netrophilmacrophages, eosinophils, megakaryocytes, erythroid and B-lymphocyte populations in marrow populations prior to, and following transplantation to normal or pretreated mouse recipients. These cultures were also used to monitor the production and purification of the specific regulatory factors necessary to stimulate the proliferation of these cells.

Progress: A cloning technique was developed that permits colony formation _in vitro_ by multipotential hemopoietic cells from mouse fetal tissues and adult marrow. Analysis of mixed colony populations showed low levels of colony forming cells and spleen colony-forming cells. These results give a simple new method for monitoring hemopoietic stem cells, possibly of a special subtype, that will have many applications in marrow transplantation studies. B-lymphocyte development in irradiated recipients following transplantation was monitored by the _in vitro_ agar cloning technique for B-lymphoid cells. B-lymphocyte precursors were shown to arise surprisingly early in hemopoietic repopulation and appear to be more closely related to erythroid precurors than previously believed. The spleen and lymph nodes in irradiated recipients were shown to be more efficient microenvironments supporting new B-lymphocyte formation by transplanted cells than the bone marrow. A selective culture system was developed for discriminating between transplanted normal and neoplastic B-lymphocytes in the mouse.

Significance to Cancer Research: The present studies are designed to develop more effective procedures for transplantation of marrow cells in leukemic patients and in patients with solid tumors undergoing autologous marrow reinfusion following chemotherapy and/or irradiation.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $87,373

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor and Delayed Type Hypersensitivity in the Mouse

Principal Investigator:	Dr. J.F.A.P. Miller
Name/Address	Walter and Eliza Hall Institute
Performing Organization:	Victoria, Australia

Contract Number: N01-CB-63992
Starting Date: 3/1/76 Expiration Date: 2/28/78

Goal: To determine whether a state of specific cellular immunity can be conferred on naive (nonsensitized) mice by subcellular material extracted from lymphoid cells of appropriately sensitized mice. Such transfer is possible in humans, and the active principle has been termed "transfer factor" (TF). The lack of a suitable animal model has been a major obstacle in characterizing TF chemically and functionally.

Approach: An objective and sensitive radioisotopic method was developed to measure delayed type hypersensitivity (DTH) in mice. Subcellular material from lymphoid cells of sensitized mice was tested for its ability to transfer specific sensitivity to naive mice.

Progress: Before any serious attempt can be made to prepare and evaluate TF in mice, certain fundamental aspects of DTH had to be examined. Highly specific DTH responses in mice could be transferred by T lymphocytes. Certain T-cell subsets were responsible for induction and transfer, whereas others, sensitive to cyclophosphamide, were involved in suppressing DTH. Certain genes in the major histocompatibility complex (MHC) on chromosome 17 influenced DTH responsiveness and transfer. Subcellular extracts of lymphnodes and spleens of sensitized mice, prepared using methods analogous to those used for human TF, failed to transfer DTH in mice. Material released from antigen-pulsed macrophages, containing both an antigen moiety and an MCH-derived component, induced potent de novo sensitization in naive mice. It is concluded that TF activity of human material depends on the presence, in the extracts, of relevant antigenic determinants and associated MHC components.

Significance to Cancer Research: Because of its overall selectivity on T cell mediated immune responses which are important in resistant to some tumors, TF-like material may be of value in immunotherapy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator:	Dr. G. J. V. Nossal
Name/Address	Walter and Eliza Hall Institute
Performing Organization:	Victoria, Australia

Contract Number: NO1-CB-23889
Starting Date: 6/30/72 Expiration Date: 10/29/77

Goal: To study the development of T lymphocytes, determine the mechanisms by which antigens influence the immunological activities of lymphocytes and to determine the cellular nature and antigenic basis of tumor-specific immune responses.

Approach: Development of T cells in organ cultures of mouse fetal thymus by cell biological and immunological techniques; study the immunological functions of B lymphocytes by serological manipulation of membrane-Ig and antigen exposure; examination of natural and induced anti-tumor activity of cells by cytotoxic activity against a series of mouse tumor lines.

Progress: After early generation of large numbers of small lymphocytes bearing the Thy 1 and TL antigens, the cultured mouse thymus produced lymphocytes with no TL and little Thy 1, but with Ly 7 and Ia antigens on their surface. Results of selective cell eliminations by cortisol treatment suggested that the two streams of lymphocyte development did not bear a simple precursor progeny relationship but could occur independently of each other. Studies on B lymphocytes showed that cells bearing only IgM were unresponsive to activating agents and susceptible to specific immunological tolerance induction compared with the majority of B cells which displayed both IgM and IgD on their surface and were readily activated to antibody production. Cytotoxic T lymphocyte-mediated immune responses induced in vivo and in vitro to a collection of mouse lymphomas and myelomas showed evidence of a number of shared and non-shared tumor antigens. Some of these were not subject to the histocompatibility antigen restriction of cytotoxic susceptibility demonstrated by others for other types of cell-bound antigens. One myeloma studied in detail was shown to possess two unique transplantation antigens. Different tumors varied considerably in their susceptibility to the action of natural cytotoxic cells and the susceptibility correlated inversely with their ability to grow in T cell-deficient nude mice.

Significance to Cancer Research: The work generally relates to development of an understanding of natural mechanisms of immunological control and potential methods of immunological intervention in cancer therapy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection and Development of H-2 Recombinant and Mutant Strains

Principal Investigator: Name/Address Performing Organization:	Dr. Donald C. Shreffler Washington University St. Louis, MO

Contract Number: N01-CB-74154

Starting Date: 3/1/77 Expiration Date: 2/28/79

Goal: The detection and fixation in congenic inbred strains of new recombinant and mutant H-2 haplotypes, particularly variants involving the I (immune response) region of the H-2 gene complex.

Approach: Screen large numbers of progeny from crosses involving different H-2 haplotypes for: (1) testing of mutagens that may enhance the frequencies of recombination and mutation; (2) screening of progeny of newly arisen recombinants for second-step recombination events; and (3) development of MLR and Ia serotyping methods for detection of I region recombinants and mutants.

Progress: During the past year, 1847 progeny from five standard crosses have been screened serologically for recombination within the H-2 gene complex. Seven recombinants have been detected. These are designated B10.BAR7, B10.BAR8, B10.BAR9, B10.BAR10, B10.ASR8, B10.KDR3, and B10.THR1. Stocks of each type are being expanded for further characterization. A second series of crosses to screen the K-S interval (instead of K-D) is currently being produced. A series of 10-15 breeding pens is currently maintained for three standard crosses, which have male heterozygous parents that have been mutagen-treated to determine effects of mutagens on recombination frequencies. To improve the efficiency and sensitivity of the MLR for future screening for I region mutants, methods have been developed that use serum-free media which reduce background incorporation levels and variability.

Significance to Cancer Research: The H-2 complex plays a critical role in susceptibility to oncogenic viruses. The new strains produced will aid in the definition of the genes that control susceptibility. The products of K, D, and I regions of the complex are involved in cell-mediated reactions against neoplastic cells. These new strains will provide material for characterization of these products.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $78,585

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of Specific Cell-Mediated Cytotoxicity

Principal Investigator: Name/Address Performing Organization:	Dr. Gideon Berke Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-74183
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Elucidation of the mechanism whereby cytotoxic T lymphocytes (CTL) interact with and kill target tumor cells (TC) *in vitro*.

Approach: Isolate and characterize a cell-free, functionally reactive component of CTL and study its mode of action on the target cells; determine the role of membrane lipid composition and microviscosity on CTL-TC interaction; do biochemcial studies on CTL-TC contact regions; evaluate the possibility that TC has an active role in the lytic process.

Progress: We have detected immunoglobulin-like "light" chains (ca. 23,000 and 25,000 daltons) in populations of immune peritoneal exudate lymphoid cells (PEL), more than 40% of which are functionally reactive CTL. The "light" chains were detected using lactoperoxidase catalyzed radioiodination followed by solubilization, immunoprecipitation, and analysis of the precipitate by PAGE. The possibility that the "light" chains were simply due to B cell contamination seems unlikely, since SIg heavy chains were not observed. Selective degradation of SIg heavy chains was excluded by deliberate contamination of PEL with minute quantities of spleen (B) cells. Macrophage-bound antibodies were also excluded, since no correlation between the degree of macrophage contamination and the amount of "light" chains was observed. Active synthesis was revealed by cultivating the cells in medium containing S-35-methionine prior to analysis. Trypsinization of the cells obliterated their binding capacity to TC and the presence of the membrane-bound "light" chains; both returned 3-5 hours after treatment.

Significance to Cancer Research: The precise understanding of the interaction of lymphocytes and tumor cells could (1) clarify the role of the immune system in the response against tumors, (2) provide reason(s) for its failure in the tumor-bearing patients, and (3) contribute to a more rational approach to immunotherapy of tumors.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $107,033

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanism by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Name/Address Performing Organization:	Dr. Irun Cohen Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-63982
Starting Date: 9/1/75 Expiration Date: 8/31/78

Goal: To learn how tumors influence the function of the immune system so that the tumor cells avoid destruction and enjoy enhanced growth.

Approach: Tumor-bearing mice develop lymphocytes in their spleens which enhance the growth of tumor cells transplanted to other mice. This enhancement is studied by measuring the effects on immune functions of tumor-enhancing spleen cells or of enhancing factor (EF) secreted by such cells.

Progress: Progress was made during the past year in identifying (a) the cells of tumor-bearing mice (TBM) which produce the enhancing factor, and (b) aspects of the host immune response affected by the enhancing factor. EF was obtained by injecting C57BL/6 mice with the syngeneic 3LL tumor. About 16-18 days later, suspensions of spleen cells of the TBM were incubated *in vitro*, and the cell-free medium was tested for enhancing activity by injecting syngeneic mice with 3LL tumor cells. EF was assayed by measuring enhancement of tumor growth produced by test medium compared to that produced by control medium. In addition to enhancing the growth of a variety of mouse tumors, injection of EF markedly increased the production of antibodies of both IgG and IgM types. Further analysis indicated that EF increases memory induction on the level of B cells, but expression of a preexisting memory state is not affected. T helper activity was found to be unaffected by the EF. These results do not necessarily indicate that EF does not act on T cells in general, since we found that EF does delay allograft rejection. Recent studies demonstrated that EF is produced and secreted by the spleen lymphoid subpopulations which do not seem to include mature T cells.

Significance to Cancer Research: Study of the mechanisms by which tumors avoid immune destruction could indicate how to control them; the immune response of some cancer patients might be directed to attack and rid the patients of cancer cells.

Project Officer: Mrs. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $39,450

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Name/Address Performing Organization:	Dr. Michael Feldman Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-63979
Starting Date: 9/1/75 Expiration Date: 8/31/78

Goal: To develop methods for in vitro sensitization of lymphocytes against autologous human malignant gastrointestinal tumors.

Approach: Seek optimal culture conditions for growth of tumor cells and for the generation of anti-tumor cytotoxic lymphocytes. In particular, the effects of different concentrations of sera autologous, allogeneic and xenogeneic to the lymphocytes, and the effects of tumor cell denisities at the sensitization and the effector phases will be tested.

Progress: Using mouse tumors as experimental models, we found that in vitro sensitization of lymphocytes against synegeneic tumors in cultures containing sygeneic serum (in contrast to the usual use of xenogeneic serum), resulted in (a) generation of cytotoxic lymphocytes (CL) of strict antitumor specificity and (b) cytotoxic lymphocytes revealing antigenic differences between local and metastatic cell populations. CL generated in culture against monolayers of cells from lung metastases, when injected into sygeneic animals with tumor cells, caused a significant reduction in incidence of lung metastasis. We tested pooled human AB sera, as distinct from xenogeneic sera, for the in vitro generation of CL against two human colon carcinomas and one kidney carcinoma. We found that such sera caused (a) improved growth of tumor cells in culture, (b) increased yield of CL generated in culture and (c) increased specificity of the cytotoxic effect. Further improvement of yield and specificity of CL was achieved when sera autologous to the lymphocytes were used. We tested the cytotoxic effect of identical ratios of lymphocytes to tumor cells when the latter are at different densities. We found that the immunogenicity and susceptibility of tumor cells to CL varied according to their growth phase and density. Studying sensitization under these conditions, we found that human spleen cells are a better source for the generation of CL than peripheral blood lymphocytes.

Significance to Cancer Research: Specific antitumor CL generated in culture could be applied in adoptive immunotherapy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Functional Differentiation of T Cells

Principal Investigator:	Dr. Martin Haas
Name/Address	Weizmann Institute
Performing Organization:	Rehovot, Israel

Contract Number: N01-CB-74180
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To establish methods for in vitro leukemogenesis of lymphocytes on thymus reticuloepithelial cells thorugh analysis of the role of retroviruses, identification of target lymphocytes and purification of different types of thymus reticuloepithelial cells.

Approach: Establish culture conditions for growing reticuloepithelial cell monolayers from leukemic and normal mice. The monolayer cultures will be analyzed for cell type, retrovirus production, and sensitivity to infection. The reticuloepithelial cell monolayers will be used in cocultivation experiments with lymphocytes in order to study differentiation of different types of lymphocytes and their transformation into leukemic cells.

Progress: Cultivation of young normal mouse thymocytes on thymus epithelial reticulum cell monolayers from leukemic mice (LTER monolayers) renders the thymocytes leukemic. Thymocytes thus cultured induce lymphatic leukemia in syngeneic mice inoculated with them. Using genetically marked thymocytes, LTER monolayers and recipient mice (using C57BL/6 mice congenic for Ly T cell antigens), we could show that the lymphomas produced originated from normal thymocytes that were cultivated on the LTER monolayer. Leukemic, immortal cell lines have not been directly obtained in vitro from thymocytes cultivated on top of LTER monolayers. Leukemic cell lines can be readily obtained by in vitro transplantation of the in vivo obtained lymphomas. In an attempt to develop a leukemogenesis system that can be completed wholly in vitro, we initiated extensive trials of leukemogenesis of normal bone marrow cells and fetal liver cells using leukemic TER monolayers and various new leukemogenic virus isolates. Two new types of leukemogenic virus isolates growing in vitro have been derived in order to promote our search for in vitro viral leukemogenicity. Cell lines of leukemic and normal thymus reticuloepithelial origin have been derived and are being tested for cell and virus markers.

Significance to Cancer Research: In vitro induction of lymphosarcomas, isolation, and analysis of the causative agents could lead to an understanding of the induction of spontaneous lymphosarcomas.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $100,348

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator: Name/Address Performing Organization:	Dr. Nechama Haran-Ghera Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-74151
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study genetic control of susceptibility to tumors.

Approach: Investigate the number, genetic map position, mutual relationship, and function of H-2 linked resistance genes to murine leukemia, including a study of genetic regulation of the phases in leukemogenesis.

Progress: The H-2 complex in the mouse was found to contain resistance genes to murine leukemia. There is a strong homology between H-2 and the major histocompatibility complex of other species including man. We have recently described complementing loci in the I region of the H-2 complex, which regulate sensitivity to leukemogenesis by A-RadLV, a variant of the radiation leukemia virus. The localization of these genes is identical to those of complementing immune response genes. We have performed a comparative genetic study with three different virus variants. H-2 linked resistance was found to all three, but the resistant and sensitive alleles and loci were different. Association with most of the immunologically important loci was demonstrated. The data suggest that if H-2 resistance genes have immunological functions, different processes of the immune response concertedly may be involved in the genetic regulation of resistance to murine leukemia. The heterogeneity of the resistance patterns suggest that the different virus passages may probably evoke antigenically different responses. If human leukemia involves antigenically different agents, this may result in a degree of heterogeneity in HLA-leukemia associations. It was also found that only one of the three virus passages was oncogenic in recipients carrying the H-2 type wherefrom these viruses were isolated. Selective effects of H-2 type on murine leukemia virus passages will be studied in the future, together with immunological studies of the observed resistance patterns and their involvement in the phases of leukemogenesis.

Significance to Cancer Research: (NCP Objective 2 Approach 3
Objective 3 Approach 3,4)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $96,746

CONTRACT RESEARCH SUMMARY

Title: Immunobiology of Metastases

Principal Investigator:	Dr. Shraga Segal
Name/Address	Weizmann Institute
Performing Organization:	Rehovot, Israel

Contract Number: N01-CB-74185
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Further analysis of the antigenic diversity of lung metastases generated by the 3LL tumor.

Approach: Generation of cytotoxic T-lymphocytes in culture against M-3LL and in L-3LL and cross-testing their cytotoxic effects.

Progress: Experiments performed in our laboratory revealed that the sensitization in culture of syngeneic lymphocytes against monolayers of the 3LL tumor cells, in the presence of syngeneic, instead of the generally used xenogeneic fetal calf serum, during the sensitization phase, results in cytotoxic lymphocytes (CTL) specific to 3LL. The cross reactivity with other syngeneic tumors or fibroblasts obtained when sensitization is carried out in the presence of xenogeneic serum was eliminated when lymphocytes were sensitized in the presence of syngeneic serum. We used the same approach to investigate whether cell populations of the local tumor (L-3LL) differ antigenetically from those of the tumor metastases (M-3LL). We found that CTL generated against monolayers of M-3LL were lytic to metastatic target cells of the local tumor. Such antimetastatic CTL suppressed the formation of lung metastases when inoculated with tumor cells obtained either from the local growth or from a tumor metastasis. CTL generated against cells of the local tumor (L-3LL) were unable to inhibit formation of lung metastases.

Significance to Cancer Research: The fact that metastases differ antigenically from their primary tumor ancestry may contribute to our understanding of mechanisms by which metastases avoid immune destruction by the host. In addition, these findings may lead to more rational appraches to the problem of immunotherapy and control of metastasis spread and growth.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: $52,124

CONTRACT RESEARCH SUMMARY

Title: Purified Thymic Products and Other Agents Promoting Lymphocyte Differentiation

Principal Investigator:	Dr. Byron H. Waksman
Name/Address	Yale University School of Medicine
Performing Organization:	New Haven, CT

Contract Number: N01-CB-43926
Starting Date: 6/17/74
Expiration Date: 11/15/77

Goal: Obtain and purify "lymphocyte activating factor" (LAF) from various types of macrophages and investigate kinetics of LAF production, improved conditions of production, and mechanism of LAF action on mouse and rat T cells.

Approach: Survey a variety of normal cell populations containing macrophages and continuous macrophage-like cell lines for production of LAF. Study various methods of culture, kinetics of LAF production, and techniques of purification. Test effect of LAF on different types of target lymphocytes and different parameters of response (macromolecular synthesis, lymphokine production, cytotoxicity) and on cell cycle. Use pharmacologic agents to analyze mechanism.

Progress: LAF or LAF-like activity (mitogenic for murine thymocytes) has been obtained from human and murine monocytes and from several adherent cell lines in culture: P-388 D1, J774-1, PU-5, WEHI-3. Activity has been demonstrated in molecules with molecular weights of ca. 13,000 and 80,000. At least 10 purification techniques have been evaluated. By use of hollow fiber devices, isoelectric focusing, and polyacrylamide gel electrophoresis, up to 1500-fold purified, active LAF can be obtained.

Rat macrophage-derived LAF has been shown to act on stimulated rat T cells only during the late G_1 phase of the cell cycle, to be insensitive to cyclic AMP level, but to depend on cAMP-sensitive events during the G_0-G_1 step of lymphocyte triggering.

Significance to Cancer Research: Macrophage products like LAF are essential participants in triggering the T lymphocytes responsible for specific immunity in cancer. A full knowledge of LAF's chemistry and biological action is needed to permit us to influence the induction of tumor immunity.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Synthesis of Cord Factor-Like Components

Principal Investigator:	Dr. Walter J. Gensler
Name/Address	Boston University
Performing Organization:	Boston, MA

Contract Number: NO1-CP-65783

Starting Date: 6/30/76 Expiration Date: 8/31/78

Goal: Synthesis of 6,6'dimycolythrehalose (or cord factor) as well as a group of α-alkyl-β-hydroxy-carboxylic acids modeled after mycolic acid.

Approach: Synthetic routes directed to the goals have been devised and are being followed.

Progress: The synthesis of meromycolic acid has been completed, and experimentation is now in progress on the remaining steps to 6,6'-dimycolytrehalose. Samples of the acid as well as the ester have been submitted for testing. A total of nine compounds related to mycolic acid have been synthesized and submitted.

Significance to Cancer Research: As soon as authentic synthetic cord factor of known structure is available, tests can be carried out with it to determine its activity as an immunomodulating agent in tumor control.

Project Officer: Dr. Herbert Rapp

Program: Tumor Immunology Program

Technical Review Group: Committee on Cancer Immunotherapy (C&P)

FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules

Principal Investigator:	Dr. Helen Van Vunakis
Name/Address	Brandeis University
Performing Organization:	Waltham, MA

Contract Number: NO1-CP-23243
Starting Date: 4/20/72 Expiration Date: 1/31/78

Goal: To develop immunoassays that can be used to determine carcinogens, procarcinogens, cocarcinogens, and their metabolites in physiological fluids, tissue extracts, and other material. Such assays also facilitate studies on pharmacokinetics, metabolic conversions, and are useful in the characterization of enzyme systems.

Approach: The compound is coupled to a macromolecular carrier that promotes its antigenicity and the resulting conjugate is used for immunization of animals. Resulting antibodies are used to devise radioimmunoassays.

Progress: Radioimmunoassays have been developed for the detection of picomoles of nicotine and its metabolites (cotinine and γ-(3-pyridyl), γ-oxo-N-methylbutyramide), the NMN, NAD, and NADP analogues of nicotine and cotinine, polycyclic aromatic hydrocarbons (benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene); mycotoxins (aflatoxin B_1); the polychlorinated insecticides (dieldrin and aldrin), and the antineoplastic agents (adriamycin, daunomycin, vinblastine, and vincristine). Nicotine and cotinine analogues of NAD and NADP were isolated and identified from incubation mixtures containing nicotine or cotinine, NAD or NADP, and a source of NADase. With radioimmunoassays specific for nicotine and cotinine nucleotides, the existence of nicotine and cotinine analogues were detected in lung, liver, and brain of rats injected with nicotine. Recently, radioimmunoassays have been developed for the carcinogens N^6 (methyl-nitroso) adenosine and N-nitroso nornicotine. The immunodominance of the N-nitroso group indicates that the immunological approach will provide important analytical procedures to detect the presence and/or formation of nitroso compounds under physiological and chemical conditions.

Significance to Cancer Research: In addition to monitoring levels of noxious agents in the environment, the availability of such assays facilitates basic studies on carcinogens, procarcinogens, and cocarcinogens that may help us understand how they exert their biological activities.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 126,421

CONTRACT RESEARCH SUMMARY

Title: Tumorigenesis in Tolerant Guinea Pigs

Principal Investigator: Name/Address Performing Organization:	Dr. Jerome R. Pomeranz Case Western Reserve University Cleveland, OH

Contract Number: N01-CP-43354
Starting Date: 2/15/72 Expiration Date: 6/30/78

Goal: (1) To study the role of carcinogens with both contact sensitizing and carcinogenic properties on the induction of tumors in animals that have been previously sensitized or rendered tolerant to the carcinogen by a variety of means. (2) To study the induction of primary mammary tumors following the infusion of DMBA into guinea pigs and to investigate the effects of complete Freund's adjuvant (CFA) administration at different times, and doses on the induction of tumors in guinea pigs.

Approach: Tolerance was induced by multiple gastric feedings of dimethylbenz (α)-anthracene (DMBA). Sensitization was accomplished by topical application of DMBA. Mammary tumors were induced by infusion of DMBA.

Progress: Studies on the effect of contact sensitization and immune tolerance to DMBA on oncogenesis in guinea pigs have been completed. When 20 mg DMBA was injected, mortality, presumably due to toxicity, was very severe. However, among survivors, there was little or no difference in the tolerized, sensitized, or control groups. When 7 mg was injected, tolerized animals had a roughly comparable rate of tumor formation to controls. Data for sensitized groups are not available.

The observation that femoral vein infusion of guinea pigs induces mammary tumors which have been interpreted as adenocarcinomas has been reconfirmed. The latency period of the tumors in Hartley strain animals was about one year. In DMBA infused (20 mg) adult female strain 2 guinea pigs with induced mammary adenocarcinomas, the latency period was approximately 14 months. Attempted transplatation of these tumors has not been successful. The mammary tumors induced in the strain 2 guinea pigs are histologically similar to those found in the Hartley strain guinea pigs.

Significance to Cancer Research: The studies outlined above represented a direct approach to the investigation of the immune response following the administration of a chemical carcinogen, and might be relevant to clinical situations in human medicine.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Colonic Tumors in Guinea Pigs

Principal Investigator: Name/Address Performing Organization:	Dr. Gary L. Cockerell Cornell University Ithaca, NY

Contract Number: N01-CP-65744

Starting Date: 9/30/76 Expiration Date 10/31/78

Goal: To produce carcinomas of the colon in approximately 700 NIH guinea pigs and subsequently test the immunotherapeutic effect of BCG vaccines versus other modalities of treatment.

Approach: N-methyl-nitrosourea is administered by intrarectal installation to produce colonic carcinomas. Following tumor induction, laparotomies are performed, and the tumorous portion of bowel is either: 1. injected intralesionally with BCG vaccines, 2. surgically resected and a diverting colostomy established, or 3. located but not otherwise treated. Animals are then allowed to recover from surgery and are observed until death.

Progress: The biohazard containment facilities necessitated by the use of carcinogen in the final stages of completion, and the tumor induction period will start soon. In the meantime, several preliminary studies have been conducted:

1) The effect of BCG vaccine in the descending colon of the normal guinea pig has been studied. Administered subserosally, the vaccine produces a severe, focal, transmural granulomatous colitis which resolves over a period of 5 months. Guinea pigs remain completely asymptomatic throughout this period.

2) Surgical technique for tumor resection has been perfected. End-to-end or side-to-side anastomosis of the guinea pig descending colon results in colonic impaction proximal to the suture line; animals invariably die within a week of surgery. A diverting colostomy technique has been developed in which the colon is transected, the distal stump tied off or removed, and the proximal stump sutured to a stoma created in the ventral midline incision. This procedure results in about an 80% success rate, and the colostomy has continued to function in animals observed for as long as 5 months post-surgery.

Significance to Cancer Research: The guinea pig colon carcinoma model will serve as a useful system to test various methods of tumor treatment, since it has now been shown that the guinea pig colon tolerates intramural BCG injection and is surgically resectable.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: $166,810

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Spontaneous Mammary Tumors

Principal Investigator: Dr. Etienne Y. Lasfargues
Name/Address Institute for Medical Research
Performing Organization: Camden, NJ

Contract Number: N01-CB-74201
Starting Date: 9/29/77 Expiration Date: 9/28/78

Goal: To develop immunologic means for prevention of the development of spontaneous neoplasms.

Approach: Cell surface antigens isolated by 3M-KCL extraction from normal and neoplastic mouse mammary cell lines are administered by several routes to determine whether these materials will influence the development of spontaneous mammary tumors in high tumor incidence strains of mice. The effects of irradiated whole cells and of cell surface antigens from primary cultures will be compared.

Progress: BALB/cfC3H mice have been inoculated by the intrademic, intramuscular and subcutaneous routes with cell surface antigens extracted from a BALB/cfC3H tumor and BALB/c normal mammary cell lines. Protein doses equivalents of 25 and 50 μg are inoculated per mouse at 3 time intervals; Freund adjuvants are added to all intramuscular and subcutaneous inocula. A new tumor cell line C57fRIII was established from which cell surface antigens are being prepared: a goal of 30 mg protein equivalents was set in order to carry on this proposal. Sufficient amounts of antigens have been accumulated from normal BALB/c, C57Bl and BALB/c ST spontaneous tumor cell lines to insure that all cell line antigens will be on test, as scheduled by the end of the first year. Early data based on the statistical evaluation of hyperplastic nodules will begin to be available in July. The part of the project to follow will be to test the protective activity of irradiated cells while accumulation of surface antigens from primary cell lines will proceed. The unexpected difficulties in accumulating sufficient amounts of surface antigens from primary cells will delay their inoculation to the end of the second year.

Significance to Cancer Research: Possibility of vaccination for man.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: $68,000

CONTRACT RESEARCH SUMMARY

Title: Procurement of Sulfolipids from Mycobacterium tuberculosis Strain H37RV

Principal Investigator: Name/Address Performing Organization:	Dr. Mayer B. Goren National Jewish Hospital Research Center Denver, CO

Contract Number: N01-CP-65746
Starting Date: 6/8/76 Expiration Date: 6/7/78

Goal: To define the role of cord factor (P3) in oil in aqueous saline emulsion antitumor vaccines and to develop simpler substitutes.

Approach: Sulfatide III of M. tuberculosis and synthetic pseudo cord factors act as substitutes for cord factor in immunotherapeutic vaccines. It is hypothesized that the role of cord factor is physical; that acting as an amphipathic, it provides the means for incorporation of endotoxin in the oil phase of the emulsion. Simpler amphipathic compounds are being prepared.

Progress: Earlier studies were devoted to recovery of the rare SL III from M. tuberculosis, and they demonstrated the impressive antitumor activity of SL III and of simpler synthetic pseudo chord factors. The relatively abundant SL-I-SLIII have now been found to have excellent activity in the line 10 systems as substitutes for cord factor. The targets for synthesis are molecules structured on a central polar core substituted with two or more large lipophilic "tails." Eight potential candidates have been synthesized and are currently being screened for their ability to potentiate the pulmonary granulomagenicity of Re endotoxin, an indicator of potential activity. The direct acylation of Re endotoxin with p hexadecyloxyphenyl butyric acid has given us preparations that are fully active in the line 10 system without requiring any added cofactors. This is potentially the most important result for the present period, for it provides the strongest support for the proposed mechanism of action P3, while the acylation may be anticipated to diminish the toxicity of endotoxin.

Significance to Cancer Research: These studies are defining the role of cord factor in antitumor activity, and are expected to provide inexpensive and readily exploitable substitutes for this expensive, exotic natural product.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Antigenicity of Precancerous Lesions in Animal Models

Principal Investigator:	Dr. Richard G. Olsen
Name/Address	Ohio State University
Performing Organization:	Research Foundation Columbus, OH

Contract Number: N01-CB-74159
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To determine whether precancerous cells carry antigenic markers which are distinguishable either in type or concentration from those exhibited by normal or by malignant cells.

Approach: Using the Rickard strain of feline leukemia virus (FeLV) in SPF cats, study will be made of the sequential development of feline oncornavirus associated cell membrane antigen (FOCMA) in preleukemic bone marrow cells in cats infected with FeLV. Correlations will be sought with other markers associated with the preleukemic phase of the disease.

Progress: Indirect immunofluorescence on parrafin embedded tissue sections was used to determine the distribution of feline leukemia virus group-specific antigens in tissues of cats infected with feline leukemia virus. Intracytoplasmic antigen was present in intestinal crypt epithelium, bone marrow, spleen, lymph node, and intestinal lymph nodules. This study has identified the tissues where preneoplastic lesions occur.

Using an indirect immunofluorescence assay specific for feline oncorna-virus-associated cell membrane antigen (feline leukemia tumor antigen) tumor antigen was detected as early as 3 weeks post-infection on bone marrow cells. By 4 weeks virus post-infection both bone marrow and mesenteric lymph node cells were expressing tumor antigen. Tumor antigen is apparently occurring in preneoplastic cells by 4 weeks post-infection. This precedes tumor induction by at least 6 months. In addition to the above studies, a very sensitive radioimmune assay (paired radio iodine labled antibody test) has been developed which permits the detection of tumor antigen on in vitro infected cells as early as 24-48 hours post-infection.

Significance to Cancer Research: The immunologic rejection of tumor tissue is recognized as the pivotal factor in the control of cancer. These studies will determine whether qualitative and/or quantitative differences in tumor antigen expression on the early preneoplastic lesion influences the subsequent course of tumor development.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: $72,000

CONTRACT RESEARCH SUMMARY

Title: Chemical Carcinogenesis and Immunity

Principal Investigator: Name/Address Performing Organization:	Dr. Bruce Zwilling Ohio State University Research Foundation Columbus, OH

Contract Number: N01-CP-53329
Starting Date: 3/25/75 Expiration Date: 9/30/78

Goal: To test the hypothesis that chemical carcinogens may also influence the immune capacity of a host and allow the potential tumor cell to escape immune surveillance.

Approach: During the course of treating rats with methylnitrosourea or the noncarcinogenic analog diphenylnitrosamine, antibody levels to teichoic acid as well as several parameters of lymphocyte and macrophage function were assessed in animals not overtly stimulated with antigen.

Progress: Treatment with carcinogen did not appear to alter the majority of immunologic parameters studied. Some alterations occurred in natural antibody levels, spleen weight, and in peripheral blood differentials of rats which had received the highest carcinogen dose (4.5 mg/kg). These alterations, however, appeared to coincide with the development of tumors and were not observed in animals with premalignant lesions.

Subsequent studies using 9.0 mg/kg MNU have confirmed our initial findings that levels of carcinogen that induce tumors do not necessarily result in suppression of the immune response. When the higher carcinogen dose was used, tumors began to appear at the 20th week. Changes in immunological reactivity coincided with tumor appearance. Teichoic acid antibody levels increased as did spleen weight. The peripheral blood lymphocyte decrease also occurred at this time and appears to be primarily a decrease in T cells. The response of macrophages to chemotactic stimuli increased in both the sacrifice and serial studies. The response to MIF appeared to decrease.

Significance to Cancer Research: This work will provide insight into basic alterations in effector cells of the immune system resulting from persistent exposure to low levels of chemical carcinogens.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Tumors in Rabbits

Principal Investigator: Name/Address Performing Organization:	Dr. John W. Kreider The Pennsylvania State University Milton S. Hershey Medical Center Hershey, PA

Contract Number: NO1-CB-74158
Starting Date: 9/29/77 Expiration Date: 9/28/78

Goal: To develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant to the malignant stage.

Approach: Using the Shope papilloma in the rabbit, a naturally occurring model of neoplastic progression, attempts will be made to intervene in the progression from the preneoplastic stage using nonspecific (BCG, _C_. _parvum_) and specific (tumor cell vaccines) immune stimulants.

Progress: Shope papilloma is a naturally-occurring animal model of neoplastic progression. The tumor, induced by a virus, is initially a benign papilloma, and about 25% of these spontaneously regress. Most of the surviving tumors eventually become metastasizing epidermoid carcinomas. Regression or progression in this system is the result of the success or failure of an immunologically mediated tumor rejection. We have attempted to intervene in the pathogenesis of the tumor by the administration of a vaccine two days after the challenge papillomas were initiated at other sites. The vaccines contained allogeneic papilloma tissue, either alone or combined with various doses of BCG. The cell vaccine, alone and in combination with 10^7 CFU significantly retarded the growth of challenge papillomas. Vaccine combined with 10^6 or 10^8 CFU was ineffective.

In a separate study, intratumoral injection of BCG cell walls on oil droplets produced extensive destruction of the injected papillomas, but the tumor mass recovered from this loss and resumed the usual growth rate. The papillomas were slightly but not significantly smaller than the controls. Neither treatment produced an increased frequency of regressions. The most important aspect of the study is whether the incidence of carcinomas will be altered by the treatments. Since the malignancies usually appear after 1-2 years, it is not yet possible to draw any conclusions.

Significance to Cancer Research: (NCP Objective _1_ Approach _3,4_)
Objective _2_ Approach _1,4_)

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Chemical Carcinogenesis and Immunity

Principal Investigator: Name/Address Performing Organization:	Dr. Abraham G. Osler Public Health Research Institute of the City of New York New York, NY

Contract Number: N01-CP-55679
Starting Date: 3/25/75 Expiration Date: 3/24/78

Goal: To study the effects of repeated exposure to low doses of a chemical carcinogen on the host's natural antibody and complement levels.

Approach: Sprague-Dawley rats received biweekly feedings of one of three dosages of the chemical carcinogen N-nitrosomethylurea for a period of about 9 months. A fourth group of untreated rats served as controls. The animals were bled at periodic intervals for serum assays of hemolytic antibody and complement component activities.

Progress: Sequential blood specimens obtained from the Sprague-Dawley rats subjected to biweekly feeding of three levels of a carcinogen, as well as from the control groups were analyzed for several component levels of the complement system. Analysis of the data comprising several hundred specimens indicate that carcinogen feeding exerted no consistent effect on the variables under study. With the exception of an infrequent aberrant finding, feeding of the carcinogen N-nitroso, N-methyl urea or of the non-carcinogenic analogue diphenylnitrosamine, yielded no significant changes in the levels of the hemolytic complement system or several of its components. Therefore, it appears that repetitive administration of this carcinogen over a period of many months did not seem to influence the process of carcinogenesis or those facets of the host's immune system, which might be influenced by the complement system.

Significance to Cancer Research: Understanding of the mechanism of carcinogenesis may offer clues to its prevention.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Malignant Melanoma in Guinea Pigs

Principal Investigator: Name/Address Performing Organization:	Dr. Wallace Clark Temple University School of Medicine Philadelphia, PA

Contract Number: N01-CP-33200
Starting Date: 10/1/72 Expiration Date: 12/31/77

Goal: The induction of malignant melanoma in the guinea pig in order to study the developmental biology of a primary neoplasm in relationship to metastases.

Approach: This inductive agent was DMBA applied to the backs of both ears and both flanks. The animals were photographed at regular intervals, and all stages of development were studied by photography, histology, and fine structure.

Progress: Malignant melanoma has been induced in several guinea pig strains. The development of the induced changes in the epidermal melanocytic system has been documented over a time span of 2-150 weeks. The tumors are characterized by widespread metastatic disease, and their developmental biology is analogous with a number of human and animal tumor systems.

Significance to Cancer Research: Provides a dependable model for the induction of malignant melanoma in an animal model.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies on an In Vivo/In Vitro System as a Potential Bioassay

Principal Investigator: Name/Address Performing Organization:	Dr. Robert Strunk University of Arizona Tucson, AZ

Contract Number: N01-CP-65778
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To investigate and standardize this in vivo/in vitro guinea pig macrophage system using a series of known chemical carcinogens run in parallel with non-carcinogenic analogues.

Approach: Young adult Hartley guinea pigs (GP) are injected intramuscularly (IM) with known chemical carcinogens, their non-carcinogenic analogues, or solvent controls. A peritoneal exudate (PE) is induced in each GP by intraperitoneal (IP) injection of 7 ml of a sterile 3% starch solution 48 hours after the IM injection of a carcinogen at a dose equivalent to 50% of the LD50. Ninety-six hours after the IP injection, PE cells are harvested, washed, and tested for capacity to synthesize the second (C2) and fourth (C4) components of complement (C). Total protein synthesis is also measured.

Progress: Dimethylnitrosamine (DMN), and its noncarcinogenic analogue, diphenylnitrosamine (DPN), were used to establish the appropriate methods for detection of carcinogenicity. C2 and C4 synthesis by PE cells collected 144 hours after injections of DMN was decreased by 40 to 50% when compared to synthesis by control cells, whereas PE cells from DPN injected animals was decreased by only 10%. The effect was maximal when the carcinogens were injected 48 hours prior to the induction of the PE. Ten other known carcinogens were tested using this system. Injection of none of these compounds was associated with depressions of both C2 and C4 synthesis. Depresssion of C2 was observed after injection of Aflatoxin B_1 and Benzidine; depression of C4 was observed after injection of $BeSO_4$. Several compounds caused increased synthesis of both C2 and C4. Acute toxicology parameters and toxic pathology were determined for each compound.

Significance to Cancer Research: Development of an assay system for the identification of carcinogenic compounds will enable us to avoid contact with substances having this property.

Project Officer: Dr. Tibor Borsos and Dr. Virginia Dunkel
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunoprophylaxis of Cancer Eye in Cattle

Principal Investigator: Name/Address Performing Organization:	Dr. Stephen J. Kleinschuster Utah State University Logan, UT

Contract Number: N01-CB-74155
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: The goal of this research is to develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant (benign) to the malignant state and to relate the immunologic status of experimental animals to clinical events.

Approach: Intralesional administration will be made of BCG cell wall vaccine into benign ocular lesions of cattle to determine whether such treatment will retard or prevent the normal progression of these lesions to the malignant state. Additionally, the immunologic status of animals will be monitored via PPD skin testing in an attempt to correlate immunocompetance with regression, arrest or progressive disease.

Progress: One hundred and twenty animals (of an ultimate total of 240) bearing benign ocular lesions have been collected and randomly assigned to the following groups: active vaccine, sham vaccine, surgical controls, and untreated controls. Within each group of animals, half have lid lesions and the other half globe lesions. Prior to initiation of treatment, all animals were found to be PPD negative. Treatment has been initiated, and blood components (RBC, sera, and lymphocytes) are being collected monthly for subsequent study.

Significance to Cancer Research: Studies involving the immunologic interruption of the transformation of a benign precursor lesion to one of malignancy at the earliest possible stage provide valuable contributions to the overall cancer effort. Additionally, via a program of monitoring the immunocompetance of experimental animals as related to clinical events following experimentation, relevant information can be contributed to the establishment of effective immunologic testing needed in order to evaluate therapy and provide guidelines for improved human clinical protocols.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 78 Funds: $311,500

CONTRACT RESEARCH SUMMARY

Title: Procurement of Large Volumes of Sera from Cancer Patients

Principal Investigator:
Name/Address
Performing Organization:

Dr. John J. Lynch
Bio Med, Inc.
Washington, DC

Contract Number: N01-CB-74125
Starting Date: 9/30/77
Expiration Date: 9/29/78

Goal: Collection of 200 ml of plasma from donors with gastrointestinal cancer (36), melanoma (10), lung cancer (25), and breast cancer (25) as well as control specimens for use in immunodiagnositc studies

Approach: Collect clinical data at the time of plasmapheresis. Store plasma in aliquots at -70°C or ship to specified recipients.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 3, 5)

Project Officer: Ms. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $71,592

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator:	Dr. Maria Linder
Name/Address	California State University
Performing Organization:	Fullerton, CA

Contract Number: N01-CB-74119
Starting Date: 11/1/76
Expiration Date: 7/31/78

Goal: The enzyme activity and concentration of (CP) are elevated in animals with tumors and in humans with lung cancer. The goal of this contract is to determine whether this phenomenon prevails for humans with other kinds of cancer, and whether tests of CP would be useful in early diagnosis, for prognosis, or in treatment of the disease.

Approach: The primary approach is to measure CP antigen concentrations, enzyme activity, and plasma Cu concentrations in large panels of sera from cancer activity, and plasma Cu concentrations in large panels of sera from cancer patients and controls, examining different kinds of cancer, different stages of the disease, patients before and after treatment or recurrence, and in the case of lung cancer, heavy smokers who may develop the disease.

Progress: For lung cancer patients we have clearly shown that plasma CP and Cu concentrations are elevated above the normal and above levels found in patients with non-malignant lung disease or heavy smokers. The most important finding is that the degree of elevation is related to prognosis: (a) it correlates with disease stage, and (b) with patient survival; and (c) after successful treatment, levels return to normal. In lung cancer patients, CP also correlates with recurrence, persistent elevations of CP antigen being indicative in 84% of cases surveyed. Initial occurrence of lung cancer also correlates with an increase in CP, but here measurement of enzyme activity is somewhat more sensitive. Measurements of CP and plasma Cu are not useful in diagnosis of breast cancer or its prognosis. Although concentrations are above normal, the same is true for plasma from patients with non-malignant breast disease. We are currently completing studies on patients with gastrointestinal cancer.

Significance to Cancer Research: Our findings so far indicate that measurements of CP are useful in following the progress of patients with lung cancer and in predicting the severity of the disease. It also may be useful as one of a series of assays used in early diagnosis of this disease.

Project Officer: Ms. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committe on Cancer Immunodiagnosis
FY 78 Funds: $14,010

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Monocyte Function in Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Ralph Snyderman Duke University Durham, NC

Contract Number: N01-CB-74121
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To determine the significance of abnormal monocyte function in humans with cancer.

Approach: The relationship between the clincal status of cancer patients and their monocyte chemotactic responsiveness *in vitro* is being determined. In addition, other parameters of phagocytic cell function are being examined to determine the specificity of depressed monocyte chemotaxis in cancer patients. The effects of serum, malignant effusions, tumor extracts and urine from cancer patients on phagocytic cell function *in vitro* are being studied. The effect of tumor removal on phagocytic cell function is being examined.

Progress: During the present year, we have extended our follow-up on 56 patients with melanoma previously tested for abnormal monocyte chemotaxis (CTX) before therapy; after an average follow-up of 40 months, 70% of the patients who originally showed normal CTX are still alive, while 65% of the patients with abnormal CTX have died from or experienced a recurrence of their disease.

Examination of neutrophil chemotaxis in 43 new patients with solid tumors revealed no defects. Two other monocyte functions, adherence to plastic and phagocytosis of antibody-coated sheep erythrocytes, were also examined in those patients. Both functions were normal or enhanced. Extracts of human renal adenocarcinoma tissue caused inhibition of monocyte chemotaxis and enhancement of adherence *in vitro*. Pleural fluids from patients with intrathoracic neoplasms inhibited monocyte chemotaxis *in vitro*.

Significance to Cancer Research: The production, by neoplastic cells, of a substance which adversely affects monocyte function could be decisive in preventing the immunological elimination of cancer cells. Characterization of such a substance could have important implications with respect to both detection of cancer and therapeutic intervention.

Project Officer: Ms. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $85,445

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator:	Dr. George L. Wright, Jr.
Name/Address	Eastern Virginia Medical Authority
Performing Organization:	Norfolk, VA

Contract Number: NO1-CB-53955
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: Identify, purify, and characterize tumor-associated antigens from renal and transitional cell carcinomas for the purpose of developing sensitive immunodiagnostic assays and assays for monitoring the cancer patients' specific anti-tumor immunity.

Approach: Tumor-associated antigens are extraced from fresh surgical tumor tissue and cultured tumor cells, and the biological activity of these crude extracts and purified fractions, obtained by physicochemical procedures, are measured by a leukocyte migration inhibition test.

Progress: Renal cell (RCC) and transitional cell carcinoma (TCC)-associated antigens were detected in 3M KCl solubilized extracts of surgical tumors, and the transitional cell carcinoma cell line: T24, as determined by the inhibition of leukocyte migration. Between 60-82% of the cancer patients reacted to the disease related crude antigen prepration, while 22-36% of patients with other tumors reacted. After antigen purification by gel filtration and gradient acrylamide gel electrophoresis, the frequency of reactivity remained approximately the same in the disease related cancer patients but the cross reactivity was considerably reduced in patients with other tumors and benign diseases. This suggests that a normal or benign tissue antigen was separated from the tumor-associated antigen.

Significance to Cancer Research: The availability of purified and well characterized tumor antigens would greatly facilitate the development of more sensitive immunoassays for diagnosis, therapy, and management of the cancer patient .

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committe on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays for Circulating Tumor-Associated Antigens: CEA

Principal Investigator:	Dr. Theodore Hersh
Name/Address	Emory University
Performing Organization:	Atlanta, GA

Contract Number: N01-CB-43887
Starting Date: 3/15/74 Expiration Date: 3/14/78

Goal: To determine the clinical usefulness of carcinoembryonic antigen (CEA) and other tumor-associated antigens, such as CEA-S, as adjuncts in the differential diagnosis of gastrointestinal cancer.

Approach: Patients with gastrointestinal symptoms had blood drawn for CEA level and other tumor-associated antigens. The clinical work-up was accomplished as is usual in order to arrive at a specific diagnosis. At the end of the year, the patients were clinically re-evaluated.

Progress: Over 1,600 patients have been enrolled. Blood has been sent to the reference laboratories for CEA and CEA-S assays. The patients were re-evaluated at a year's anniversary to verify the initial diagnosis. Preliminary results are being obtained from the computerized data base on 1,156 patients enrolled in the study; 79% had an initial diagnosis of a benign condition, 13% had a malignancy, and in 8% no definitive diagnosis was established. There is a significant overlap between the CEA values of patients with initial benign and malignant diagnoses. Values found for CEA-S indicated that 96.8% of cases with benign disease were in the normal range, compared to 81.5% of those with cancer. For CEA, 10 of 872 (1.1%) of cases with benign diagnoses had values above 20 compared with 23 of 129 (17.8%) patients with malignancy.

Significance to Cancer Research: (Preliminary) There is a considerable overlap of normal and elevated CEA values in cases with benign and malignant conditions of the gastrointestinal tract. This lack of specificity does not make the CEA assay a useful adjunct in the differential diagnosis of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0 Site Visit Date: 12/2/75

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Adly N. Ibrahim Georgia State University Atlanta, GA

Contract Number: N01-CB-84267
Starting Date: 2/1/78 Expiration Date: 1/31/79

Goal: Immunodiagnosis of cervical and head-and-neck cancers.

Approach: Antisera are prepared in rabbits against cervical cancer tissues. Elimination of antibodies against normal tissues and normal human sera are carried out by various methods. These antisera will be used for the detection of circulating tumor-associated antigens (C-TAA) in sera of patients with cervical and head-and-neck cancers. Serum controls including those from normal individuals and from patients with benign diseases of these sites will be used.

Progress: Antisera against crude and partially purified CaCx are being prepared in rabbits. These antisera, after adsorption, detect circulating tumor associated antigens in cancer patients. Antisera will be accumulated for purification.

Cervical cancer tissues are being accumulated to prepare antigens to start purification and isolation of purified TAA to be used for preparation of antisera.

Significance to Cancer Research: Results obtained, so far, suggest the possibility of developing serological tests for diagnosis, prognosis and monitoring patients with cervical and head-and-neck cancers.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $70,601

CONTRACT RESEARCH SUMMARY

Title: Tumor-Specific Antigens in Diagnosis and in the Management of Cancer Patients

Principal Investigator:	Dr. T. M. Chu
Name/Address	Health Research, Inc.
Performing Organization:	Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-33858
Starting Date: 6/25/73 Expiration Date: 10/24/78

Goal: To study the relationship of CEA in the evaluation of recurrence and survival for cancer patients with curative surgery.

Approach: Examine the prognostic value of the preoperative and follow-up CEA in relation to other preoperative variables; study the distribution profiles of CEA at surgery, during follow-up period and at recurrence; implement a plan for patients whose risk of recurrence is greatest based upon preoperative and serial CEA; develop predictive models which integrate the CEA levels with other significant prognostic variables to determine the probability of recurrence in order to recommend adjuvant therapy.

Progress: A long-term follow-up of prognostic value of the CEA has been studied in 74 patients with resectable colorectal cancer. Twenty-nine developed recurrence of disease (median: 20.4 months), 25 patients died (median: 22.0 months). A discrete data analysis indicated that the preoperative CEA was a significant indicator of disease recurrence. Based upon various Statistical analyses, no significant correlation was shown between CEA and Dukes' classification. However, the combination of CEA and Dukes' was more significant as a prognostic parameter than either indicator alone. Patients with Dukes' C tumor and an elevated preoperative CEA had the worst prognosis. It was shown, by scattergram techniques and t-test, that concommitant chemotherapy did not affect follow-up CEA levels. Further, matched pairs and scattergram techniques indicated the CEA rose at reccurrence from preoperative levels and CEA did not rise from preoperative levels at fixed times during disease free period. Right ratio analysis is being used to develop a decision algorithm to predict recurrence. Another group of 130 resectable lung cancer patients is also available for evaluation.

Significance to Cancer Research: CEA is a laboratory assay which can be used as an aid in the management of certain cancer patients.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $66,081

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Use of Leukemia-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Jun Minowada Health Research, Inc. Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-74165
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: Study the expression of leukemia-associated antigens in leukemia patients at the time of disease presentation, and at appropriate intervals during and after first remission induction.

Approach: Study the expression of leukemia-associated antigens in leukemia non-T/non-B ALL; human thymus-leukemia antigen; and blast-leukemia-associated antigen.

Progress: A total of 186 leukimia samples were studied for cell surface markers. These samples included 121 blood-bone marrow specimens for patients with acute lymphoblastic leukemia (ALL), 19 specimens from patients with acute myelocytic leukemia (AML), 11 specimens from patients with chronic lymphocytic leukemia (CLL), 18 specimens from patients with chronic myelocytic leukemia (CML) and 16 specimens from patients with other types of hematologic malignancies. The immunological marker determination was able to identify positively subtype of ALL (non-T, non-B ALL, T-cell ALL and B-cell ALL), CLL and "lymphoid" type of CML in blast crisis. The assay was also used negatively to suggest some types of leukemias, such as AML, "myeloid" type of CML in blast crisis and other hematological malignancies. By monitoring the presence of leukemia-associated antigens, approximately 7% of the patients with ALL in complete remission on the basis of conventional criteria have shown by this immunodiagnositc assay to have a residual leukemia (at a level of 1% antigen-positive cells) in the the bone marrow.

Significance for Cancer Research: Immunological subclassification provides the basis for better treatment of leukemia of an individual patient.

Project Officer: Mrs. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $67,389

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Name/Address Performing Organization:	Dr. Leonard Weiss Health Research, Inc. Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-53901
Starting Date: 6/1/75 Expiration Date: 5/31/79

Goal: Investigation of the macrophage electrophoretic mobility assay and tests deriving from it, as a valid tool in the diagnosis and monitoring of malignant disease.

Approach: The original MEM test is dependent on the electrokinetic response of guinea-pig macrophages to lymphocyte-products from patients. These macrophages are tedious to obtain, difficult to measure, and vary from animal to animal. We have therefore tested formaldehyde-fixed, tannic acid-treated sheep erythrocytes in place of macrophages in the FTEEM test.

Progress: In patients with a history of cancer, positive results were obtained in 28/42 cases with the FTEEM test compared with 32/42 in the MEM test. In people with no history of cancer, negative results were obtained in 16/18 cases with the FTEEM test, compared with 12/18 in the MEM test in the present series, and 51/69 in a more extensive series, based on a blind study organized by NCI.

Significance to Cancer Research: This assay could be potentially useful in monitoring the clinical status of patients with, or with a history of, cancer rather than in initial detection of the disease. It could also provide a method of examining the apparently important role played by macrophage-lymphocyte-tumor interactions in the progress of malignant disease. Depending on the medical criteria used, both the MEM and FTEEM tests give unacceptedly high levels of either false positive or false negative results for screening or general diagnostic purposes.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator:	Dr. John Fenimore Cooper
Name/Address	Kaiser Foundation
Performing Organization:	Oakland, CA

Contract Number: N01-CB-74169
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: Evaluate an immunodiagnostic test for prostatic cancer.

Approach: Accumulate and assess clinical oncologic data for prostatic cancer with a solid phase radioimmunoassay technique for prostatic acid phosphatase (RIA-PAP); continue development of the RIA-PAP technique as a screening technique in the early diagnosis of prostatic cancer utilizing a large random population of elderly males.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 5 Approach, 1,4,7)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $96,087

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Antibodies to Melanoma

Principal Investigator: Name/Address Performing Organization:	Dr. Peter Hersey Kanematsu Institute Sydney Hospital Sydney, Australia

Contract Number: N01-CB-74120
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Determine whether there is a correlation between the clinical course of melanoma growth and levels of serum antibodies.

Approach: Employ lymphocyte dependent cytotoxic antibody assays.

Progress: 48 patients with localized melanoma have been entered into the study in the 5 months of the present contract period. Initial results confirmed previous findings that patients can be grouped according to whether they have LDA against melanoma cells or not. Those with melanoma LDA can be subdivided further into those in which the LDA activity varies in relation to clinically determined melanoma growth and those in which the LDA activity appeared unrelated to melanoma growth. A small proportion of the apparently unreactive sera had LDA activity in the IgG fraction. The study period is too short yet to attempt any correlations between melanoma growth and the various patterns of melanoma LDA activity. The findings of different patterns of melanoma LDA activity in patients provides the opportunity of determining whether the clinical course of melanoma can be related to LDA activity. The finding in some patients that variations in the level of melanoma LDA appear to vary with tumor growth underline the possible importance of these assays in immunodiagnosis melanoma.

Significance to Cancer Research: (NCP Objective 5 Approach 4,5)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $44,299

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigens

Principal Investigator:	Dr. Calvin A. Saravis
Name/Address	Mallory Institute of Pathology
Performing Organization:	Boston, MA

Contract Number: N01-CB-64071

Starting Date: 6/16/76 Expiration Date: 5/31/78

Goal: To develop an assay system for the tumor marker, ZGM, that is useful in the diagnosis and management of digestive tract malignancy.

Approach: To further refine a radioimmunoassay for detecting ZGM in the blood of patients with gastrointestinal cancer and make a clinical evaluation of it comparing it to CEA.

Progress: Avid high titer anti-AGM antisera were produced to purified ZGM and used to analyze tumor and normal tissue extracts. ZGM was detected in primary and metastatic colon cancer and was not detected in normal human plasma, normal colon, normal liver, and normal spleen. It was distinct from CEA, from antigens, cross-reacting with CEA, AFP, alpha-2 macroglobulin, ferritin-like molecules, blood group antigens, and buffy coat. ZGM showed no reaction in a radioimmunoassay for CEA. During the present contract year a ZGM radioimmunoassay was developed. The ZGM-RIA measured less than 2.5 ng of ZGM standard. RIA analysis of both ZGM and CEA activity in the same plasma specimens obtained from patients with benign diseases, large bowel polyps, colonic malignancy and noncolonic malignancy, gave different and complementary findings.

Significance to Cancer Research: The detection of the ZGM in patient specimens may permit earlier diagnosis, better evaluation of prognosis and monitoring of therapy for cancer.

Project Officer: Ms. Judith M. Whalen

Program: Tumor Immunology Program

Technical Review Group: Committee on Cancer Immunodiagnosis

FY 78 Funds: $101,499

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests

Principal Investigator: Name/Address Performing Organization:	Dr. Allan Schutt Mayo Foundation Rochester, MN

Contract Number: N01-CB-23879
Starting Date: 6/15/72 Expiration Date: 11/14/78

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the Tumor Immunology-Immunodiagnosis Program.

Progress: The Mayo Serum Bank is a central storage area for a large number of blood samples, including those collected by Immunodiagnosis contractor at the Philadelphia Geriatric Center and the University of Minnesota cancer screening facilities, aside from collection of samples. The Mayo Bank also ships panels of serum samples to investigators under the Immunodiagnosis Program's direction to evaluate in a blind fashion potential immunodiagnostic tests for cancer. Test panels have been sent to a total of 122 investigators since the establishment of the bank. Extensive data are kept on all samples and continuous efforts are made to increase the inventory of samples from patients with benign or malignant diseases to supply adequate numbers of samples to screen immunodiagnostic tests, as they are developed, for potential use in clinical cancer diagnosis.

Significance to Cancer Research: This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $90,609

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Use of Cross-Reacting Microbial Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Christine H. Granatek M.D. Anderson Hospital and Tumor Institute Houston, TX

Contract Number: N01-CB-74122
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To study the distribution of an isolate microbial crossreactive antigen in human leukemia and melanoma, produce specific antisera, and develop a radioimmunoassay for detection of circulating crossreactive antigen.

Approach: Initial screening of tumor cells by immunoflourescence, and of cancer patients' sera by a solid phase RIA, to detect reactivity with antimicrobial sera. Isolation of crossreactive components by affinity chromatography. Specificity testing by absorption studies and cross-testing in RIA, with normal donor and benign disease patient control.

Progress: Recent studies have demonstrated immunological crossreactivity between Mycobacterium bovis (BCG) and human malignant melanoma and acute myelogenous leukemia cells, and these observations were confirmed in our laboratory. We have primarily been concerned with determining the extent and specificity of the observed crossreactivity, since these factors are crucial to any future immunodiagnostic trials. The data suggest that the immune response of these patients to BCG is via tumor-crossreactive components. Antisera prepared in rabbits against M. bovis or M. plei CWS, or against E. coli endotoxin, S. tryphimurium Re mutant endotoxin, or re detoxified glycolipid, have all been shown to crossreact in a solid phase RIA. Cross-absorptions with tumor cells are in progress. Affinity chromatography of 3M KCl extracts of human tumor or nomral tissue vs. antibody to BCG produced 19% and 13% yields of crossreactive antigen from melanoma and leukemia, respectively; while only 0.8% and 0.3% of protein was crossreactive in normal liver and peripheral blood leukocytes.

Significance to Cancer Research: Assays based on either detection of circulating antigen or antibody may be useful in clinical diagnosis or monitoring of patients with these malignancies.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $89,961

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes

Principal Investigator: Name/Address Performing Organization:	Dr. J.A. Loos Netherlands Red Cross Amsterdam, The Netherlands

Contract Number: N01-CB-74164
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Find the optimal conditions for cryopreservation of human monocytes.

Approach: Analyze the following variables: purification methods, concentration of cryoprotectant, controlled cooling rate, the thawing procedure, and dilution and subsequent removal of the cryoprotectant. Once optimal cryopreservation is achieved, functional studies will be done.

Progress: The first item was to make a choice between two monocyte purification methods, i.e., isolation by selective glass adherence or separation by buoyant density. Both methods gave 80% purity, but the yield of the separation by density was 45% in comparison with 80% obtained by glass adherence. The functional properties like oxygen consumption during phagocytosis or chemotactic response to caseine were significantly higher in monocytes obtained by separation on the basis of density. The recovery after freezing in liquid nitrogen, storage for 12 hours and thawing was 98 ± 4% (n = 3) for monocytes obtained on the basis of density and 78 ± 3% (n = 14) for glass adherent monocytes. The post-freeze decrease of the functional properties like chemotaxis and oxygen comsumption was significantly higher in the monocytes separated by buoyant density centrifugation. These results suggest that isolation of monocytes on the basis of density selected a population which was not as representative for the total as glass adherent monocytes. Therefore the latter purification method was chosen for further studies. So far it may be stated that by the conventional cryo-preservation method for lymphocytes the post freeze recovery and survival of monocytes is as good as that of lymphocytes. Trypan blue stainability and cell volume were not changed by the freezing and chemotaxis and oxygen consumption during phagocytosis were only slightly decreased.

Significance for Cancer Research: (NCP Objective 4 Approach 1,2)

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $49,191

CONTRACT RESEARCH SUMMARY

Title: Clinical Application of Assays for Tumor-Associated Antigens

Principal Investigator:	Dr. Clement C.S. Hsu
Name/Address	Northwestern University
Performing Organization:	Evanston, IL

Contract Number: N01-CB-74132
Starting Date: 9/26/77 Expiration Date: 9/25/78

Goal: To investigate the clinical applicability of rabbit antisera, anti-EP and anti-ALL, prepared in our laboratory and the clinical relevance of cell-bound blocking component on leukemic lymphocytes.

Approach: By serial examination of lymphocytes from patients with lymphocytic leukemias during the course of illness and patients with other diseases for the anti-EP and anti-ALL reactivity.

Progress: The anti-EP has been found to recognize B-lymphocyte-associated (or human Ia-like) antigen which is present on normal B-lymphocytes, monocytes, chronic lymphocytic leukemia (CLL) cells and acute lymphoblastic leukemic (ALL) cells. The anti-ALL identifies a membrane antigen which seems to be specific to ALL cells. Our preliminary study also revealed that antiserum reactivates with leukemic lymphocytes may be inhibited in some patients due to cell-bound blocking component. The component may be removed form the cell surface by incubating the cells overnight at room temperature in Roswell Park Memorial Institute Medium 1640. We have thus far examined 60 ALL patients, 25 CLL patients, 30 patients with other leukemias, 31 non-leukemic patients and 25 normal individuals, using the indirect immunoflourescent technique. Important results are: 1) Anti-ALL reactivity is limited to ALL cells with an exception of CLL cells from a single case; 2) ALL cells from 9 out of 60 cases did not react with anti-ALL; and 3) In 4 out of 9 CLL cases and 6 out of 20 ALL cases, the blocking component was demonstrated on leukemic cell surfaces.

Significance to Cancer Research: Our results indicate the usefulness of anti-ALL in differential diagnosis and subclassification of ALL. Further studies of cell-bound blocking component may lead to understanding of another mechanism by which leukemic cells evade recognition and destruction by host immunological apparatus.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $67,325

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Arthur Malley Oregon Regional Primate Research Center Beaverton, OR

Contract Number: N01-CB-53954
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: Isolate homogenous preparations of tumor-associated antigens from human melanoma and squamous cell carcinoma tissue in sufficient quantity to permit biological and chemical characterization.

Approach: Tumor tissue is extracted, purified, and tested for the presence of tumor-specific antigens by dermal skin tests and leukocyte adherence inhibition.

Progress: The specificity of the LAI assay was examined by testing various tumor extracts with leukocyte suspensions of tumor patients and normal healthy donors. A linear relationship exists between the concentration of protein in a melanoma extract giving a 10 mm indurated skin reaction and the concentration of protein giving a maximum LAI reaction. A highly purified melanoma antigen preparation was obtained by carboxymethyl-cellulose chromatography followed by preparative isoelectrofocusing with a pH 5 to 10 ampholine gradient. Additional studies to determine the relationship of β_2-microglobulin and melanoma tumor antigens (MTA) revealed that MTA binding to an anti-β_2-microglobulin adsorbent was not due to the presence of HLA antigens, and was specifically blocked by free β_2-microglobulin but not BSA or NHS. Attempts to demonstrate tumor-specific antibodies in rhesus antimelanoma sera or rabbit antimelanoma sera (obtained from Dr. Reisfeld) have been unsuccessful. Current efforts are directed at using the partially purified melanoma antigen preparation to make antimelanoma antigen antibodies that can be used as a diagnostic tool.

Significance to Cancer Research: The availability of pure tumor antigens will provide a reagent that will permit the development of sensitive in vitro radioimmunoassays for the early detection of tumor antigen. In addition, the structural characterization of these pure tumor antigens may provide some insight on how tumor antigens escape the immune apparatus of the host. Structural modification of the pure tumor antigens may permit their use in enhanced cellular immunity against the determinant that activates T cells.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Name/Address Performing Organization:	Dr. Henry Altschuler Philadelphia Geriatric Center Philadelphia, PA

Contract Number: N01-CB-33914
Starting Date: 6/25/73 Expiration Date: 10/24/78

Goal: To collect serial serum specimens from populations at high risk of contracting malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Demographically stable populations at high risk of developing human malignancies in the colon, lung, breast, prostate, bladder, pancreas, etc. will be followed on an annual basis. This includes clinical evaluation and serum collection. The sera will be banked and records kept on the patients so that information obtained from the stored sera can be correlated with development of any particular cancer.

Process: Sera are being continually collected from a demographically stable population of high cancer risk aged residents of the Philadelphia Geriatric Center as well as from others. Complete records are being kept on each patient, including medical information and follow-up data from such sources as autopsies and Tumor Board Conferences. The collected sera are being sent to Mayo Clinic for storage under Dr. Schutt's project. As of February 28, 1977, a total of 2,816 serum samples were collected, and 2,759 such samples were shipped. During this period there has been a total of 251 deaths, of which 41 were autopsied. In this same period 40 subjects have been developed malignancies. Of these, 23% have G.I. cancers. In order of decreasing frequency are cancers of skin, breast, and prostate. In addition there are a small number of miscellaneous malignancies. It should be pointed out that the population under survey is predominantly female.

Significance to Cancer Research: Early detection of cancer by means of appropriate testing would permit treatment of cancer at the most favorable time.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $58,568

CONTRACT RESEARCH SUMMARY

Title: Detection of Tumor-Specific Antigens in the Circulation

Principal Investigator:	Dr. Gerald N. Callahan
Name/Address	Scripps Clinic and Research Foundation
Performing Organization:	La Jolla, CA

Contract Number: N01-CB-74126
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To correlate the appearance and quantity of circulating tumor-associated antigens with the stage of development of the tumor and tumor site.

Approach: To detect and quantitate tumor associated or tumor-specific antigens (TAA) present in sera of mice bearing transplantable carcinomas or sarcomas. To correlate the appearance and quantity of circulating TAA with the stage of development of the tumor. Produce highly specific antisera directed toward TAA to aid in detection of these antigens.

Progress: We have obtained a total of 12 transplantable murine sarcomas and carcinomas induced with physical and chemical agents. These tumors have been expanded and large numbers of cells from early transplant generations and tissue culture pasasages have been banked in liquid N_2 for future use. Four of the tumors have been established in continuous culture. Twelve rabbits are currently being injected with both untreated tumor cells and subcellular fractions of tumor cells. These rabbits have begun to produce cytotoxic antibodies, and the specificity of these antisera is being investigated. Also syngeneic mice are being injected with irradiated or iodoacetamide treated cells. Significant prolongation of survival following tumor challenge (15-30 days over MST) have been observed, and these sera are being evaluated for antibodies reactive with tumor cells.

Significance to Cancer Research: The answers gathered in this system should allow for at least a first step in the assessment of the feasibility of this approach to immunodiagnosis of human neoplasia.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $78,932

CONTRACT RESEARCH SUMMARY

Title: Evaluation of an Isomeric Species of Carcinoembryonic Antigen (CEA-S)

Principal Investigator:	Dr. Robert M. Nakamura
Name/Address	Scripps Clinic and Research Foundation
Performing Organization:	La Jolla, CA

Contract Number: N01-CB-53934
Starting Date: 5/16/75 Expiration Date: 5/15/78

Goal: CEA-S is a specific isomeric species of carcinoembryonic antigen which is found predominantly in tumors of gastrointestinal tract origin and can be detected in trace quantities in fetal and normal body fluids. The work scope of the contract is to isolate and characterize CEA-S from colon carcinomas; to produce specific antisera to CEA-S; and develop an optimally specific and sensitive standardized assay system for CEA-S as a potential adjunct to the diagnosis of gastrointestinal malignancy.

Approach: Isolation of CEA-S ligands from several colonic carcinomas; comparison of the ligands against a panel of sera with various diseases including gastrointestinal tumor, normal sera, inflammatory bowel disease, and renal diseases; production of the antisera to CEA-S and conventional CEA; and determination of the contribution of potentially interfering glycoproteins to the CEA-S assay such as normal cross-reacting glycoprotein (NCA).

Progress: CEA-S has been isolated for 13 colonic adenocarcinomas, each exhibiting individually different patterns of isoelectric isomers; the ligands have been compared with different antisera against panels of 44 to 65 test sera, normal serum panels, and tissue extracts; from the pools of antisera; absorptions have been examined and found to differ for different antisera; non-specific cross-reacting antigen (NCA) has been isolated, assay established and results have been compared; and panels of sera from Dr. T. Hersh and M. Chu are being assayed and results returned for analysis. Current results of separate studies suggest a significantly lower incidence of false positive results for direct serum assay of CEA-S than for indirect or direct assay of CEA.

Significance to Cancer Research: The serum CEA-S assay will be a more standardized assay, more easily and less expensively performed, and provide greater specificity for gastrointestinal tumors than the conventional CEA assay. The CEA-S assay would appear to provide a helpful diagnostic adjunct in the evaluation of patients with bowel cancer and aid in the differential diagnosis of bowel cancer versus inflammatory bowel disease.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Ralph A. Reisfeld Scripps Clinic and Research Foundation La Jolla, CA

Contract Number: N01-CB-53899
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: Development of new methods for the purification and physicochemical characterization of human tumor-associated antigens and to make them available for cell-mediated immunity assays and for diagnostic testing.

Approach: Human melanoma-associated antigens (MAA) are isolated by mild, least denaturing procedures from cultured melanoma cells and their spent culture media. This includes removal of HLA antigens, lipids, purification, and physicochemical characterization of MAA which are defined biologically by their ability to evoke specific cell mediated and humoral immunity reactions.

Progress: Effective purification procedures developed include fractional salting out, ultracentrifugal flotation in potassium bromide, cation exchange chromatography, affinity chromatography on lectin columns (lens culinaris and ricin) and preparative isoelectric focusing. Purified antigens elicited delayed cutaneous hypersensitivity responses in melanoma patients as well as leukocyte adherence inhibition. Humoral immune responses were documented by inhibition of antibody binding to cultured melanoma target cells as determined in a newly developed radioimmunometric assay. Xenoantisera to purified MAA were produced in rabbits and rendered functionally specific by selective adsorptions with human red cells and cultured human lymphoid cells. These antisera were evaluated by a newly developed radioimmunometric antibody binding assay. This assay, when used as a binding inhibition test, effectively assessed the serologic activity of MAA, and was used to monitor purification. Purified MAA isolated from spent culture media has an isoelectric point of pH 6.4 and is a glycoprotein with a molecular weight of 58,000, which is not associated with β_2-microglobulin.

Significance to Cancer Research: Availability of purified MAA should prove advantageous to establish their utility to produce specific xenoantibody and to test the efficacy of these reagents for tumor diagnosis, therapy and in monitoring response to therapy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $166,470

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator:	Dr. Argyrios N. Theofilopoulos
Name/Address	Scripps Clinic and Research Foundation
Performing Organization:	La Jolla, CA

Contract Number: N01-CB-53952

Starting Date: 5/16/75 Expiration Date: 5/15/78

Goal: Detection and quantitation of immune complexes (ICs) in sera of patients with malignancies; determining prognostic significance of ICs in monitoring these patients; identification and possible isolation of the tumor antigens in ICs; and production of antisera against tumor antigens in ICs.

Approach: Levels of ICs will be determined by the Raji cell radioimmunoassay. ICs will be isolated using Raji cells or insolubilized conglutinin as substrates. Isolated antigens or ICs will be used for the production of antisera in experimental animals.

Progress: Raji cells were used for the isolation of complement fixing antigen-antibody complexes from serum. ICs bound to these cells were radiolabeled at the cell surface using lactoperoxidase and then recovered. The antigen and antibody moieties of the complexes were isolated by dissociating sucrose density gradient centrifugation or by SDA-PAGE electrophoresis. For example, these techniques were used to isolate and identify the antigens in ICs in the serum of rabbits with chronic serum sickness and rats with Moloney virus induced sarcomas. These complexes were found to contain gp70 and p30, the envelope and core antigen respectively of C-type viruses. Repeated challenge of rabbits with whole Raji cells with bound complexes or eluates from such cells resulted in antibody production against the antigens of the ICs, although reactivity against cellular and serum components were also elicited. Monospecific antisera against the antigens in ICs was produced by immunizing rabbits with the alum precipitated antigen isolated on SDS-PAGE.

Significance to Cancer Research: Techniques for identification and quantitation of ICs may help in monitoring the course of the disease and the effect of treatment. Isolation of tumor antigens and production of antisera against them will be useful in understanding the pathogenesis of malignancies and in developing diagnostic procedures.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Stuart Schlossman
Name/Address Sidney Farber Cancer Center
Performing Organization: Boston, MA

Contract Number: N01-CB-43964
Starting Date: 5/16/74 Expiration Date: 5/15/78

Goal: To develop the technology for the fractionation, quantitative isolation, and characterization of subsets of human T, B, and Null cells and monocytes from normal individuals and patients with cancer.

Approach: The development of *in vitro* methods for the characterization and identification of human classes by cell surface markers; the development of new techniques for the isolation of highly purified subclasses of human lymphocytes utilizing affinity chromatography, rosetting, and fluorescence activated cell sorting, and the utilization of *in vitro* techniques to discriminate the functional properties and interactions of isolated subsets of lymphocytes.

Progress: The contractor has focused on the isolation of subsets of human T, B, and Null cells utilizing classical cell surface determinants and those newer ones developed in his laboratory including HTL, Ia-like, TH1, TH2, and JRA. The analysis of these surface determinants has aided work in the development of new techniques for the isolation of highly purified subclasses of human lymphocytes and permitted the development of new *in vitro* techniques to discriminate the functional behavior and interactions of these isolated populations. These studies demonstrate that human T subsets have direct regulatory effects on immunoglobulin secretion or other T cell functions and are analogous to the Ly1 subset in the mouse. The similarities between the JRA$^+$ and the TH2$^+$ subsets of T cells in man suggest the preservation of suppressor cell populations in ontogeny, since these classes of cells were shown to exert suppression on immunoglobulin synthesis and other T cell functions.

Significance to Cancer Research: The isolation and characterization of subsets of cells with distinct regulatory function will have an important role in patients with cancer, since the manipulation of the host's response to tumors requires an understanding of the mechanisms by which an autochthonous immune response is generated.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator: Name/Address Performing Organization:	Dr. Carl Feit Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: NO1-CB-74124
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Collect serial serum specimens from cancer patients and benign disease controls for evaluation of potential immunodiagnostic tests for cancer.

Approach: Collect serial specimens from patients with melanoma, ovarian carcinoma, lung carcinoma, and Hodgkin's disease, plus suitably matched patients with benign skin lesions, benign adnexal masses, a history of heavy smoking, and benign lymphadenopathy to serve as controls.

Progress: In the first 6 months of this contract attention has been given to establishing effective liaison with the 4 surgical services at Memorial Hospital that are referring patients with the designated malignancies and with the Strang Clinic/Preventive Medicine Institute which serves as the source of controls. Recordkeeping procedures have been devised which make it possible for investigators using this collection of sera to correlate their findings with clinical status without further need for abstraction or tabulation of clinical data. Patient entry has started. There are now 30 persons being followed. Cooperation from both physicians and patients has been excellent. In the coming year emphasis will continue on systematic patient and control recruitment with careful and detailed followup.

Significance to Cancer Research: The effective evaluation of the prognostic value of immunodiagnostic tests requires testing sera from patients and controls which are well matched and have been collected in a serial prospective fashion. Establishing collections of such sera will expedite the evaluation of new immunodiagnostic assays as they become available.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $95,621

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Jorgen E. Fogh Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-43854
Starting Date: 8/8/73 Expiration Date: 8/7/78

Goal: To further develop procedures for encouraging tumor cell growth in culture and to perform tests necessary to define standard cell lines from various tumors.

Approach: Human tumor cell lines are established in culture from surgical specimens and are collected from other investigators. They are characterized in this laboratory as to malignancy, cell type, and special features as needed for individual investigations.

Progress: Our program includes 401 cultured human tumor cell lines. Malignant characteristics of the cultured cells, consistency of tumor cell type with tumor of origin, and special features of individual cell lines are assessed. The methods for characterization have provided additional information on cell morphology, nutritional requirements, growth in vitro, tumor production in nude mice, chromosomal characteristics, poliovirus susceptibility production and isozyme patterns. Only a very small proportion of the lines are suspected of HeLa cell contamination. Isozome analysis includes 14 enzymes to distinguish cell lines contaminated with other lines and to define uniqueness of isozyme patterns for as many lines as possible. Based upon the frequency products of phenotype combinations, one group, in addition to HeLa-like lines, is highly suspected for extraneous cell culture contamination. Isozyme phenotypes show great stability when compared under various in vivo and in vitro situations. Mycoplasmas and other contaminants have been eliminated from all but 14 lines, still mycoplasma contaminated. Three hundred and eighty-four lines and many early cultures are frozen in liquid nitrogen and, when sufficiently characterized, available to collaborating investigators. Computerized data files are being constantly expanded. Cell lines and pertinent information on cultivation methods and characterization have been provided to many investigators in the United States and abroad.

Significance to Cancer Research: The collection of well characterized contamination-free human tumor cell lines is pertinent for many disciplines of experimental and clinical cancer research.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $247,214

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth O. Lloyd Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-64021
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: The aim of this project is to purify human ovarian cancer antigens which can be detected using heterologous antisera and to develop sensitive assays for these antigens. These assay systems will then be used to detect the antigens in sera of women with ovarian cancers and of suitable controls to determine their usefulness in the diagnosis of ovarian cancer.

Approach: The approach entails the use of heteroimmune sera to detect and assay tumor-associated antigens in ovarian tumors and sera from cancer patients.

Progress: Investigations on two antigens (OVC-1 and OVC-2) defined by heterologous sera prepared against extracts of human ovarian tumor extracts have continued. OVC-1, which was found in 48 of 93 ovarian cancers, has been shown to be a new pregnancy-associated antigen. Partial purification of this antigen has been achieved; it exists in two forms having molecular weights of about 200,000 and 30,000 respectively; the larger form has a reduced subunit size of 60-80,000 daltons. OVC-2, which was found in 8 of 93 ovarian cancers, has been identified as a new fetal antigen. It has been purified to a high degree of homogeneity and is a glycoprotein with a molecular weight of about 45,000 daltons. Purified OVC-2 is being used to prepare high titered, specific antisera suitable for use in radioimmunoassays. Both OVC-1 and OVC-2 have recently been found in amniotic fluid and this may well serve as a convenient source of antigen in the future.

Significance to Cancer Research: Ovarian cancer has a high death rate because diagnosis and surgical removal are often critically delayed due to its deep location and lack of early symptoms. Immunodiagnostic assays are aimed at the early detection of ovarian tumors in asymptomatic women.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $78,811

CONTRACT RESEARCH SUMMARY

Title: Serologic Assays with Purified Melanoma-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth O. Lloyd Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: NO1-CB-74127
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and characterize cell surface antigens of human malignant melanoma and develop a sensitive serologic assay for monitoring and diagnosis of melanoma.

Progress: Autologous sera can recognize two categories of melanoma antigens: 1) individually-specific, confined to a single melanoma cell line; and 2) melanoma-specific but shared with some, but not all, allogeneic melanomas. Since these sera are too restricted in their specificities to be of general use in the immunodiagnosis of melanoma, we are developing heterologous sera directed against the antigens identified using autologous assays. Solubilization by limited papain digestion and subsequent lectin-Sepharose chromatography are effective first steps in antigen purification.

Significance to Cancer Research: New sensitive assays are needed for the immunodiagnosis and monitoring of malignant melanomas.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $63,331

CONTRACT RESEARCH SUMMARY

Title: In Vitro Augmentation of Cell-Mediated Cytotoxicity

Principal Investigator: Name/Address Performing Organization:	Dr. Hiroshi Shiku Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-74130
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To develop means to augment cell-mediated cytotoxicity.

Approach: Exposure of melanoma patient lymphocytes to autologous melanoma cells in vitro under various experimental conditions; determination of the specificity of antigens detected in cytotoxicity tests by cellular immunoadsorption and inhibition tests with fixed and unfixed tissue; and characterization of melanoma-specific effector cells as opposed to natural killer cells and suppressor cells.

Progress: Cytotoxicity of peripheral blood lymphocytes for autologous cultured melanoma cells has been detected in 12 of 20 patients. This setting permits incisive analysis of specificity not feasible with allogeneic reactions, and will be used to develop means for augmenting cell-mediated cytotoxicity. Attempts to determine optimal technical conditions for in vitro sensitization which are now in progress include allogeneic human lymphocyte-target cell combinations as well as allogeneic and syngeneic murine systems in addition to the final object of this study, autologous combinations of human cancer cells and lymphocytes.

Significance to Cancer Research: We expect this study to contribute to our understanding of cellular immunological rejection mechanisms, and to the development of methods for strengthening these mechanisms.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $93,910

CONTRACT RESEARCH SUMMARY

Title: Role of Antibody-Dependent Cell-Mediated Cytotoxicity in Tumor Immunity

Principal Investigator: Name/Address Performing Organization:	Dr. Osias Stutman Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-53894
Starting Date: 5/1/75 Expiration Date: 4/30/78

Goal: Determine the importance of cytotoxicity of syngeneic tumor cells by antibody-dependent cell-mediated mechanisms in host resistance to tumor growth or spread, and whether the presence of such antibodies would be useful for the detection of malignant or premalignant lesions and for monitoring tumor growth.

Approach: Study the role of antibody-dependent cell-mediated cytotoxicity in tumor immunity in a mouse syngeneic system using spontaneous mammary adenocarcinomas.

Progress: A reliable assay for antibody-dependent cell cytotoxicity (ADCC) using a syngeneic mouse system was developed. The antibody has activity in IgG subclasses and not in IgA or IgM; it is thymus-dependent in development and is produced by hyperimmunization with mammary tumor cells or derived from tumor-bearing C3H mice. Adherent C3H mammary tumors prelabeled with ^{3}H-proline are target cells. Effector cells are from normal C3H or C3Hf donors. Effector cells are relatively radioresistant (especially those derived from blood or peritoneal cavity; those from spleen are less sensitive); treatment of the donors with ^{89}Sr does not affect the spleen cell effectors; concerning surface markers, the effector cell is negative for all T markers while B-specific markers reduce their effectivity. The *in vitro* responses can be inhibited by relevant free antigen, excess free specific antibody, excess free irrelevant soluble Ig, aggregated or insoluble Ig, and relevant or irrelevant antigen-antibody complexes.

Significance to Cancer Research: Studies of cells involved in the immune destruction tumors are an important part of Baseline Information Flow in the Tumor Immunology Program.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Data Management System and Statistical Support for NCI Serum Panel

Principal Investigator:	Dr. Lee Richman
Name/Address	Small Business Administration
Performing Organization:	Ebon Research Systems Silver Spring, MD

Contract Number: N01-CB-74210
Starting Date: 8/8/77 Expiration Date: 8/7/78

Goal: to provide data management and statistical programming support for research projects being conducted by the Committee on Cancer Immunodiagnosis in order to determine which serum tests are best able to detect early stages of cancer.

Approach: To perform statistical analyses of investigator results on NCI serum panel evaluations and to prepare summary reports of the results.

Progress: Statistical tests, including sensitivities (the degree to which cancer victims are identified as being positive for the disease) and specificities (the degree to which those without cancer are identified as such) have been conducted on data from more than 12 researchers since last August. Serum panels from other researchers are in the process of being analyzed.

Significance to Cancer Research: Since it is important to diagnose and treat cancer as early as possible, it is important to have a diagnostic and screening tool that would enable one to detect early cases of cancer. Our analyses will provide information as to the degree of success with which researchers have developed such tools.

Project Officer: Ms. Judith M. Whalen and Mr. Michael Stump
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $53,819

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Antibodies to Syngeneic Mouse Tumors

Principal Investigator: Dr. Isaac P. Witz
Name/Address Tel Aviv University
Performing Organization: Tel Aviv, Israel

Contract Number: N01-CB-74134
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Study alterations in the serologic reactivity pattern in mice at high risk of developing carcinogen-induced or endogenous virus-induced malignancies as well as reactivity of such mice towards antigens associated with the relevant tumors.

Approach: Use the following animal model systems: spontaneous tumors in C3HeB mice; mammary tumors induced by DMBA in BALB/c mice; and lung adenomas in urethane-treated BALB/c mice.

Progress: Our breeding colonies of BALB/c and C3HeB/Fe produce at present a sufficient number of animals to set up experimental and control groups. According to the originally designed protocol, two experiments involving several hundred animals are already underway: Using a ^{51}Cr-release micro assay for complement-dependent cytotoxicity we are in the process of assaying the serologic reactivity pattern of normal BALB/c and C3HeB mice against a panel of 9 different ascites tumor target cells and against normal thymocytes. The study on the BALB/c mice is close to completion. We found that whereas normal young BALB/c mice have a low, but definite, serologic reactivity against a syngeneic plasacytoma, they have no activity against the other tumors. Since one of the basic elements of the study is a sequential bleeding of mice at various periods following experimental carcinogenic treatments, we investigate the effect of such bleedings on tumor growth (using transplanted tumor systems available in the laboratory) and on serologic reactivity. The results seem to indicate (no solid data available as yet) that sequential bleeding of mice may inhibit tumor growth to some extent and may increase titers of natural serologic reactivity. We also found that the treatment of the target cells used in the cytotoxicity assays with neuraminidase increased the sensitivity of the complement-dependent cytotoxicity microassay.

Significance to Cancer Research: The results will indicate whether or not altered serologic activity against tumor-associated antigens signals the appearance of primary or transplanted tumors.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $95,891

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-Associated Antigens

Principal Investigator:	Dr. Ilkka P. T. Hakkinen
Name/Address	Turku University
Performing Organization:	Turku, Finland

Contract Number: N01-CB-64070
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To evaluate the FS antigen (fetal sulfoglycoprotein antigen) in screening for early gastric cancer. To characterize the FS antigen and standardize the reaction, a trial mass-screening for gastric cancer is being carried out among an unselected population. Clinical material is used to test the FSA method in discriminating between benign and malignant gastric ulceration.

Approach: Acid glyccproteins from gastric juice are tested by immunodiffusion against FS-antiserum, its perparation being standarized as far as possible. Subjects analyzed as secreting FSA are examined for gastric alterations by gastroscopy, and the entire cohort followed for two years. Cancer rates will be compared with actual statistics.

Progress: More than 95% of clinical gastric cancers were earlier found to secrete FSA in gastric juice. On the other hand, less than 10% of the people over 40 secrete FSA. Results of this mass-screening trial on 53,020 subjects seem to support the hypothesis that FSA secretors form a high risk group for gastric cancer; the majority of cancers were found in the samll minority (8.7%) of FSA positive subjects. Figures for prognosis are not yet available, but there were a great many early cancers among the gastric cancers found in FSA secretors.

Significance to Cancer Research: If the method is proved to be useful, it will provide a new tool for early diagnosis of gastric cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $32,782

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Ralph E. Schrohenloher University of Alabama Birmingham, AL

Contract Number: N01-CB-53893
Starting Date: 5/1/75 Expiration Date: 4/30/78

Goal: Establish the presence of circulating immune complexes in cancer and determine the identity, levels, and diagnostic or prognostic value of these materials.

Approach: Develop sensitive and quantitative methods for soluble immune complexes where the antigen is not known, systematically screen sera from a broad cross-section of cancer patients to establish the existence of circulating immune complexes, and establish the identity of the complexes by physicochemical and immunochemical techniques.

Progress: The capacity of a monoclonal rheumatoid factor (MRF)-latex immunoabsorbent to detect immune complexes (IC) by radioimmunoassay was established using rheumatoid arthritis (RA) sera and synovial fluids. Analysis of sera from various cancers indicated the presence of IC in approximately 20% of 245 patients. Analysis by the Clq binding assay demonstrated IC in approximately 35% of the sera; however, less than 10% demonstrated IC by both the Clq and MRF assays. Further, analysis by a modification of the Raji cell radioassay indicated IC in only 7% of the sera. Both the frequency and levels of IC were less than noted for RA controls by any of the three assays used in this study. Analysis of 175 sera from 47 melanoma patients followed for up to 6 months demonstrated IC by at least one assay in one or more specimens from 29 (62%) of the patients. The relationship of the occurence of IC and clinical activity in the latter group of patients is being evaluated. These findings indicate that a spectrum of IC, as revealed by reaction with various detection systems, can occur in cancer.

Significance to Cancer Research: Studies on the occurrence of immune complexes in cancer are of potential significance in understanding the immunobiology of the disease. Procedures for immune complexes also show promise as aides in monitoring disease activity.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell-Mediated Reactivity of Normal Persons to Human Tumor-Associated Antigens

Principal Investigator:	Dr. Mitsuo Takasugi
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-74133
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: Investigate the significance of the fact that effector cells from healthy individuals and cancer patients are normally cytotoxic to cultured human tumor cells.

Approach: Examine the level and specificity of the reaction with effector cells isolated from healthy people and patients; study the level of cells bearing Fc receptors in the circulation and how these levels are affected by cancer.

Progress: Cultured tumor target cells can be classified into three rough categories designated TA 1, 2, and 3 by direct cytotoxicity and by inhibition of cytotoxicity through competition. This conclusion is now supported by studies of effector cells armed with antibodies eluted from target cells. The same effector cells which previously had exhibited natural cell-mediated cytotoxicity (NCMC) or antibody dependent cell-mediated cytotoxicity (ADCC) show a decrease in these activities when armed with the anti-SRBC. This type of blocking of NCMC occurs by replacement of natural antibodies on effector cells. ADCC is also inhibited since there are less free Fc receptors on the effector cell. The block may be reversed or unblocked with antibodies specific for the target cell. Recently, we have observed the production and release of two factors from lymphocytes in culture which enhance and suppress the level of natural cell-mediated cytotoxicity. The helper cell N activating factor (NAF) is a heat-sensitive, nondialyzable protein, which elutes from Sephadex after albumin. It is produced by T cells and enhances the activity of N cells. The suppressor has been called N cell inhibitory factor (NIF) and is being characterized at present.

Significance to Cancer Research: It appears that natural cell-mediated cytotoxicity is a cell-mediated cytotoxic system, whose specificities are directed by antibodies in the circulation. A better understanding of these processes and how cancer affects them is of great significance.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $73,990

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator:	Dr. Robert E. Gerner
Name/Address	University of Colorado
Performing Organization:	Denver, CO

Contract Number: N01-CB-74161
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Collect serial serum specimens from patients with malignant and benign diseases and "normal" individuals for NCI to evaluate immunodiagnostic tests for cancer.

Approach: Collect serial serum specimens oncancer patients before therapy and every three months following and on benign diseases and control patients. Samples are stored at -70° in 1 ml aliquots; complete clinical data are kept on patients; a minimum of 300 patients will be entered into the study.

Progress: We have purchased and obtained all necessary equipment and clinical forms to perform the study. We have interviewed 150 individuals (these include those with various malignant and benign disease plus normals) of which 75 have consented to participate in this study. From these 75 individuals, a total of 1190 1 ml vials of serum have been processed and stored at -70°C. Complete clinical data have been obtained and recorded on all of these patients.

Significance to Cancer Research: The significance of this portion of the total project is to collect, process, and make available to other investigators serial serum samples from patients with various malignancies in an attempt to develop immunodiagnostic tests for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $32,916

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Monocytes

Principal Investigator:	Dr. David Talmage
Name/Address	University of Colorado Medical Center
Performing Organization:	Webb-Waring Lung Institute Denver, CO

Contract Number: N01-CB-53900
Starting Date: 5/16/75 Expiration Date: 5/15/78

Goal: To isolate monocytes from human peripheral blood, both from normal donors and from donors having carcinomata, and to test these cells for functional activity and markers.

Approach: Mononuclear cells are being separated from whole blood; monocytes are then isolated from the lymphocytes by counterflow centrifugation using a specially designed chamber.

Progress: We have examined a number of methods of differentiating between normal lymphocytes and monocytes by light microscopy, which was considered to be desirable baceause of the large discrepancies in monocyte percentages in normal samples reported by a number of investigators. In general, those using light microscopic techniques obtain lower values than those using electron microscopy. Recently, we have obtained excellent agreement when we incubate cells on albumin-coated microscope slides in a medium containing protein and divalent cations at 37° C for 30 minutes. This procedure is designed to optimize the spreading of monocytes. At the end of this time, the cells can be fixed and stained if desired, or examined live. Phase contrast, or preferably, differential interference contrast microscopy is used. Fixing and staining with methyl green is convenient but not necessary. When phase contrast microscopy is used, the staining allows the monocytes, which are spread into an extremely thin state, to be located more easily. Using either type of microscopy, we have obtained monocyte percentages in excess of 40 from Ficoll-Hypaque separated populations.

Significance to Cancer Research: The monocyte is known to play an important role in the immune system through its ability to stimulate lymphocytes. Dysfunction of the immune response in cancer can, therefore, be examined by comparison of experiments using monocytes isolated from normal donors and cancer patients.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes for Use in Immunologic Studies

Principal Investigator: Name/Address Performing Organization:	Dr. Roy S. Weiner University of Florida Gainesville, Florida

Contract Number: N01-CB-74131
Starting Date: 9/30/77
Expiration Date: 9/29/78

Goal: To isolate human monocytes from peripheral blood and develop methods for their adequate cryopreservation.

Approach: Develop methods of collection and isolation of peripheral blood monocytes that would be applicable to normal subjects and to patients with cancer. Develop optimal techniques for cryopreservation and define the functional characteristics of cryopreserved cells. Evaluate changes in monocyte structure and function occurring in patients with cancer.

Progress: Thus far, we have established that 40% of the morphologically identifiable monocytes in 500 ml of peripheral blood can be obtained in the same 15 ml portion of the pheresis buffy coat in which 70% of the lymphocytes are obtained. The addition of 6% hydroxyethyl starch to the in-flow arm of the separation bowl disperses both lymphocytes and monocytes so that it now requires 24 ml of buffy coat to obtain 70% of the lymphocytes. Within this volume, however, 60% rather than 40% of the blood monocytes are obtained. Once again, there is no separation of monocytes from lymphocytes. Hexose monophosphate shunt activity in aliquots along the buffy coat is proportional to the concentration of monocytes, as determined by both morphology and non-specific esterase activity. We have also adapted the chemotaxis assay to human monocytes and have confirmed that monocytes obtained by pheresis are not altered in their chemotactic response from those obtained by phlebotomy. Fractions rich in monocytes from the pheresis buffy coat are currently being used to study monocyte purification by elutriation centrifugation and by adherence in order to define changes in chemotaxis, hexose monophosphate shunt activity and ultrastructure induced by adherence.

Significance to Cancer Research: We have made significant progress in collection procedures that are compatible with sequential samplings of large numbers of monocytes from patients with minimal loss of blood. We have facilitated the testing of monocyte function as it may change with anti-tumor therapy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $94,248

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigen

Principal Investigator: Name/Address Performing Organization:	Dr. Robert Humphreys University of Massachusetts Medical School Worcester, MA

Contract Number: N01-CB-64072
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To identify and isolate membrane proteins from leukemic cells in order to raise rabbit antibodies which may be used to diagnose biologic subsets of leukemias and lymphomas by cytotoxic or immunofluorescent assays.

Approach: Membrane proteins from leukemic cell lines are examined by [^{35}S] methionine, autoradiographic techniques in SDS polyacrylamide slab gel electrophoresis. T- and B-specific proteins are identified, and are then isolated by traditional protein chemical techniques. Antisera to these proteins are assayed for their specificity for leukemia or for subsets of leukemias.

Progress: p23,30 antigen from lymphoid malignancies has been isolated by raising goat anti-p23,30 serum. The distribution of p23,30 antigen on subsets of normal cells after mitogenic or allogeneic stimulation, and on leukemic cells and lymphomas is being studied. Other non HLA-A, -B and -D antigen proteins have been isolated from spleens infiltrated with lymphoid malignancies and antisera to these partly purified, and in some instances homogeneous, proteins are being raised. EBV transformed B lines from normal individuals appear similar in their patterns to Burkitt's lymphoma cells, although a few proteins appear uniquely on some cell lines. T lymphoblast cell lines are substantially different from B lines in the appearance of their membrane protein patterns. That the heterogeneity in the cell lines was not the result of variability among outbred humans was demonstrated by identity of patterns from PHA-stimulated lymphocytes from laboratory personnel.

Significance to Cancer Research: The identification of biological subsets of leukemias permits correlation to the clinical course. These assays may in time affect the selection of chemotherapeutic protocols based on the identification of biological subsets of cancers, or the antisera may be used to modify the immune response in patients with cancer.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $89,780

CONTRACT RESEARCH SUMMARY

Title: Suppressor Monocytes in Cancer Patients

Principal Investigator: Name/Address Performing Organization:	Dr. Norman T. Berlinger University of Minnesota Minneapolis, MN

Contract Number: N01-CB-74160
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To study the effects of suppressor monocytes on cellular immune functions in cancer patients and the relevance of these effects to the clinical course of the disease.

Approach: To study how frequently patients with epidermoid cancer of the head and neck show evidence of circulating suppressive monocytes and how the presence, absence, disappearance, or reappearance of these cells correlates with prognosis and the clinical course of the disease.

Progress: Nearly 50 patients with this cancer have been studied to date. The majority of them show deficits of cellular immunity, many of the magnitude seen in cogenital immunodeficiency diseases. Although follow-up so far has been short, it appears that patients with deficient cellular immunity as measured in mixed leukocyte culture seem to have poorer prognoses. In most of these patients the deficient cellular immunity seems to be secondary to a population of regulatory cells, which inappropriately exert a suppressive effect on the defensive functions of T-lymphocytes. These regulatory cells appear to be monocytes and to inhibit T-lymphocyte blastogenesis. The identity of this cell as a monocyte has been suspected by our experiments that eliminate patient monocytes from mixed cell cultures with the result of increased lymphocyte blastogenesis, and from experiments that return patient monocytes to these cultures with the effect of suppressed lymphocyte blastogenesis.

Significance to Cancer Research: A critical quantification of cellular immunity in the cancer patient could help identify those patients who are high risk for doing poorly with their disease. An understanding of the nature of the deficient immune responses could allow the development of rational immunotherapeutic modalities.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $80,639

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Name/Address Performing Organization:	Dr. John S. Najarian University of Minnesota Minneapolis, MN

Contract Number: N01-CB-33915
Starting Date: 6/30/73 Expiration Date: 4/30/78

Goal: Serum is collected from individuals in the high cancer risk age group (50 to 70 years of age) during their annual visit to a cancer detection center. The serum is coded, frozen, and stored for future evaluation of potentially promising immunodiagnostic tests for cancer.

Approach: In addition to collection of the serum samples, detailed history is kept which includes the date cancer is detected, and the date and cause of deaths of any members in the study. When the study and collection are completed, this information will be used to designate at least three groups of serum samples: those who never develop cancer; those who develop cancer within a year of the sample; and those who develop cancer before the sample is taken. When a new immunodiagnostic test of promise is developed, its potential in early detection of cancer can be validated very quickly by means of this serum bank.

Progress: Since the study started, 5,290 asymptomatic adults have been examined yearly for cancer, and serum has been collected for the study. A total of 12,000 exams have been performed and 119,007 serum aliquots coded, frozen and stored for future evaluation.

Significance to Cancer Research: The importance of early diagnosis of cancer has been repeatedly demonstrated and can result in increasing the life span of those involved. The collection of a bank of serum from individuals whose cancer histories have been carefully and accurately documented can reduce the time required to evaluate the potential of a new diagnostic test from many years to a few days. This results in tremendous savings of money, and more importantly, reduces the time required for initial determinations of value and establishments of efficacy.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection of Tumor Specific Antigens in the Circulation

Principal Investigator: Name/Address Performing Organization:	Dr. R.W. Baldwin University of Nottingham Nottingham, England

Contract Number: N01-CB-74167
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Investigate the diagnostic applicability of circulating tumor-associated antigens.

Approach: Follow the levels of circulating antigens during tumor growth in a variety of situations, attempting to relate measured levels of antigen to tumor size. A wide range of rat tumors (3-methylcholanthrene-induced sarcomas, hepatocellular carcinomas and spontaneously arising mammary adenocarcinomas in Wistar rats) will be used.

Progress: Studies with hepatoma D23 have established that tumor specific antigen in both free form and as immune complexes can be detected in the circulation during the early stage of subcutaneous or intramuscular tumor growth. By using tumor cell challenges near to the threshold dose required for consistent tumor growth it has also been established that an early peak of tumor antigen in serum can be detected at least 10 days before tumor becomes palpable. These methods will also be used to study syngeneic transplants of a spontaneously arising mammary carcinoma, Sp4, since on implantation into mammary tissue, this produces mammary tumors which eventually metastisize to the axillary nodes, so providing a model for human breast cancer (Greager and Baldwin, 1978).

Significance to Cancer Research: (NCP objective 5, Approach 4,5). These studies will establish in animal systems the value of monitoring circulating tumor antigen for diagnosis.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $50,138

CONTRACT RESEARCH SUMMARY

Title: New In Vitro Techniques to Evaluate Cell-Mediated Immunity to Intact Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Daniel Thor University of Texas Health Science Center San Antonio, TX

Contract Number: NO1-CB-43893
Starting Date: 6/1/74 Expiration Date: 7/31/78

Goal: To establish methods for measuring the cell-mediated immune response of tumor patients to their tumor antigens.

Approach: To develop and apply the agarose droplet micromethod for measuring cell migration inhibition in patients with bladder, lung, and breast carcinoma using antigens derived from autologous tumor material and from established tumor cell lines.

Progress: Development of the agarose droplet method using a murine tumor allograft rejection model has been completed, permitting rapid technical development of the method and verification of its use for testing immunity to cell surface antigens. Development of a human agarose droplet micromethod utilizing leukocyte migration inhibition has been completed and used to test the migration inhibition response of human tuberculin-sensitive individuals to PPD. Application of the micromethod to assess human tumor immunity has demonstrated positive migration inhibition reactions in bladder, lung, and breast carcinoma patients to corresponding tumor antigens. Specificity studies revealed specific reactivity among bladder cancer patients when tested with allogeneic bladder carcinoma antigens, to which they showed inhibition of migration; and in simultaneous studies with lung carcinoma antigens, no reactivity was observed. Patients carrying tumors showed significantly greater reactivity than patients with no evidence of residual tumor. Furthermore, 3 of 16 chronic genito-urinary disease patients (cystitis) and 2 of 8 renal pelvis transitional carcinoma patients demonstrated some reactivity to bladder carcinoma antigens. No leukocyte migration inhibition reactions against bladder carcinoma antigens were demonstrated in normal human controls.

Significance to Cancer Research: The accurate assessment of antitumor immunity in cancer patients may be important to both diagnostic and treatment decisions. Development of appropriate methods is critical to this effort.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-Associated Antigens

Principal Investigator: Dr. E. Russell Alexander
Name/Address University of Washington
Performing Organization: Seattle, WA

Contract Number: N01-CB-64068
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To develop and purify an antigen and antiserum which are associated with epidermoid carcinoma of the cervix. To develop methods to use that antigen and antiserum in serological diagnosis of cervical cancer.

Approach: Monospecific antiserum was prepared against the tumor associated antigen. Affinity chromatography is used to purify antigen based on monospecific antiserum, used, in turn, to detect circulatory antibody and antigen.

Progress: When pure monospecific antiserum reacts against crude tumor antigen in two-dimensional immunoelectrophoreses, a single precipitin line results. This line has been demonstrated in 31 cervical cancer tissues, absent in one, absent in 10 other gynecologic cancers and 9 normal gynecologic tissues (including seven specimins of normal cervical tissues). The antigen is clearly protein in nature, at least in regard to the major antigeneic determinant. Monospecific antiserum was utilized in examination of 39 cervical cytologic smears by an indirect immunofluorescent antibody method (20 from cervical cancer patients, and 19 from women with normal cervices). Antigen was seen clearly in both membrane and cytoplasma of cervical cancer cells in all cancer cases and no controls. Observations were repeated on 2-5 smears for patients with identical results. I^{125} labelled cancer antigen and monospecific rabbit antiserum have been used in a radioimmunassay to detect circulatory antigen in 13/20 sera from cancer patients but in only 2/20 sera from control patients.

Significance to Cancer Research: The aim of this study was to develop an immunodiagnostic method for early detection of cervical cancer, and to determine if the relationship of such findings related to the development of abnormal cervical cytology.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $116,675

CONTRACT RESEARCH SUMMARY

Title: New Methods for Isolation and Characterization of Human PBLs

Principal Investigator:	Dr. Hans Wigzell
Name/Address	Uppsala University
Performing Organization:	Uppsala, Sweden

Contract Number: N01-CB-74129
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To develop new methods to isolate and characterize human peripheral blood lymphocytes.

Approach: Obtain unique markers, morphological or functional, specific for a given subgroup of mononuclear cells by use of classical and innovative cell purification techniques and other methods.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach _)

Project Officer: Ms. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $54,000

CONTRACT RESEARCH SUMMARY

Title: Antibodies to Human Organ- or Tissue-Associated Antigens

Principal Investigator:	Dr. Jen-Fu Chiu
Name/Address	Vanderbilt University
Performing Organization:	Nashville, TN

Contract Number: N01-CB-53896
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To detect antibodies to human organ- or tissue-associated antigens to use in study of antigenic profile of normal and neoplastic cells, both from biopsy and tissue culture.

Approach: Obtain specific antibodies to chromosomal nonhistone proteins associated with homologous DNA, and measure in tumor cells the quantitative decrease or loss of normal antigens and increase of tumor-specific antigens. Distribution and expression of these antigens will be studied in relation to disease type, stage of disease, and prognosis.

Progress: To determine whether the nuclear antigens found specific for malignant neoplasms (as compared with their parental tissue antigens) may be characteristic for rapid cell proliferation rather than for the malignant phenotype, we have produced and assayed antisera to the G_1, S, and G_2 phases of the HeLa cell cycle. There was no difference in the immunoreactivity of G_1, S, or G_2 chromatins when assayed with these antisera indicating the absence of a growth-related nuclear antigen. Nude mice were utilized as a source for steady supply of well characterized and homogeneous human tumor material. Antibodies to nuclear antigens from human lung, colon, and breast carcinoma were produced. Cell specific antibodies were produced to dehistonized chromatin of human neutrophils. These antibodies recognize (by immunolocalization) various precursor cells (in bone marrow) of granulocytic lineage as well as circulating leukemic cells of granulocytic origin. Myeloblasts and cells of lymphocytic or erythrocytic lineages were not detected by these antisera.

Significance to Cancer Research: Recognition and characterization of nuclear, DNA associated antigens which are specific for cellular phenotype (normal or malignant) is essential for *in situ* detection of malignant cells.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Breast Tumor-Associated Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Robert K. Oldham Vanderbilt University Nashville, TN

Contract Number: NO1-CB-74162
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and purify the TAA of MCF-7, a breast cancer-derived tissue culture line, to generate heteroantisera for development of a radioimmunoassay for that antigen. Antigen isolation and purification is to be first analyzed with respect to antigen specificity in the leukocyte migration inhibition assay.

Progress: The first necessary phase was to demonstrate that antigen induced production of leukocyte migration inhibition factors was cryopreservable. Several leukocyte donors with positive skin tests and LMI assays to PPD have been recruited for leukophoresis. These cells will serve as a model system for the study of the cryopreservability of LMI. Women with carcinoma of the breast or in high risk categories for this disease are currently being tested for LMI to breast cancer antigens from the MCF-7 cell line and are being recruited for possible leukophoresis and subsequent cryopreservation. During this period large quantities of MCF-7 have been grown and cyropreserved for future use in antigen extraction and purification. Membranes have been isolated from these cells on a dextran-polyethyleneglycol gradient following hypotonic lysis. 3M KCl extracts of these membranes have been prepared for testing in LMI assays. Preliminary separation of the protein components of these extracts has been achieved using discontinuous polyacrylamide gel electrophoresis.

Significance to Cancer Research: Isolation of TAA and the development of a radioimmunoassay for carcinoma of the breast would be a valuable diagnostic and prognostic tool.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $108,384

CONTRACT RESEARCH SUMMARY

Title: Cell-Mediated Reactivity of Normal Individuals to Human Tumor-Associated Antigens

Principal Investigator:	Dr. Robert K. Oldham
Name/Address	Vanderbilt University
Performing Organization:	Nashville, TN

Contract Number: N01-CB-74166
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Investigate the effector cell subpopulations involved in natural cytotoxicity, the antigens to which this activity is directed and the relationship of natural activity to the immune response directed at tumor-associated antigens.

Approach: Define the natural cytotoxic activity of the K-562 model system with normal and patient populations; characterize the effector cell; investigate the model system antigens.

Progress: At this point, approximately 200 individuals have been tested in all age and sex brackets. No age-related levels of natural cytotoxicity have been noted as yet. However, there is some evidence for the existence of high responder and low responder subpopulations in all age groups. Several high responder individuals have been recruited for leukophoresis and large numbers of aliquots of their leukocytes have been cryopreserved. Also, large numbers of target cells grown in a single batch have been cryopreserved. Natural cytotoxicity has been demonstrated to be cryopreservable and cryopreserved target cells are demonstrably useful reagents in this assay. Thoracic duct lymphocytes from chronic thoracic duct drainage of prospective kidney graft recipients have been tested for natural cytotoxicity late in the course of drainage. Preliminary data on the competitive inhibition of natural cytotoxicity by membrane components of target cells have been obtained.

Significance to Cancer Research: An understanding of the mechanism and target antigen system of natural cytotoxicity is necessary for understanding the biologic significance of this phenomenon and for quantitating its contribution and relationship to *in vitro* assays of tumor immunity.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: $82,484

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Dr. John A. Pritchard
Name/Address Velindre Hospital
Performing Organization: Whitchurch, Cardiff, Wales

Contract Number: N01-CB-53956
Starting Date: 6/29/75 Expiration Date: 10/31/77

Goal: Isolate and identify an effector compound released by cancer-sensitized lymphocytes, or by stimulation with appropriate antigen; development of a refined apparatus for the measurement of macrophage mobility in the bioassay for the presence of the effector compound. Where possible, both systems to be appraised by a series of coded blood samples to establish the clinical value of the macrophage electrophoretic mobility (MEM) test for malignant disease.

Approach: Large-scale preparation of the effector compound, macrophage slowing factor (MSF), to be undertaken. The active fractions to be pooled (by ultrafiltration) and used to raise a rabbit anti-MSF serum. Assessment of the rank Mk.III electrophoresis system for the simple measurement of macrophage mobilities.

Progress: The mediator of macrophage slowing (MSF) has been found to have a molecular weight of 20-23,000 and is stable at 56° and a storage (4 weeks) at -10°C. Bioassay, using macrophage mobility reduction, showed that activity as retained at 10^{-3} dilution. The Rank Mk.III electrophoresis system has been developed and assessed in the operation of the MEM assay and found to produce similar results as obtained with the Zeiss cytopherometer with considerably less operator fatigue. The clinical value of the test was assessed in a blind trial using 210 coded blood samples from normal subjects and patients with either benign or malignant disease. The false negative rate for cancer patients averaged 43%, and the false positive rate for non-cancer patients averaged 34%, indicating that the test at the present time, under routine laboratory conditions, is not reliably reproducible and does not have the ability to effectively discriminate between benign and malignant disease.

Significance to Cancer Research: The MEM test has been proposed as a sensitive laboratory test for malignant disease. This can only be truly assessed in the routine operation of the test in conjunction with accepted clinical procedures.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator:	Dr. Dieter Burger
Name/Address	Washington State University
Performing Organization:	Pullman, WA

Contract Number: N01-CB-63990
Starting Date: 9/1/75 Expiration Date: 8/31/78

Goal: To isolate immune complexes from the circulation and identify the role which these antigen-antibody complexes may play during enhancement or regression of tumor growth.

Approach: Affinity chromatography with immobilized equine complement Clq is used as a sorbent for isolation of complement Clq-fixing immune complexes using feline leukemia as a model for human cancer. Purified immune complexes are quantitated serologically and then identified as to their specificity. These data are correlated with the clinial status of the animal, presence of leukemia group-specific antigen, and the titer or antitumor antibody.

Progress: We have isolated immune-complexes (I.C.) from cats with and without serologic evidence of feline leukemia infection and identified them as directed against feline respiratory viruses. In addition to feline leukemia membrane (FOCMA) antigens, we tested against normal feline tissue antigens and feline calicivirus and feline rhinotracheitis virus. Serum-donors were classified on the basis of clinical status and immunologic tests as: 1) Progressors (28 cats), 2) Regressors (2), 3) FeLV-carriers (20), and 4) Unrelated illnesses (11). Immune-complexes were detected in one or more animals of all these groups. Progressor animals generally had low levels of I.C. and zero values in terminal cases with non-regenerative aplastic anemia. The two regressor animals exhibited PKI/PKII ratios between high (>0.25), very high (>0.4) and low (>0.125) depending on the time of sampling. FeLV carrier animals and normal controls had levels which ranged from low to high. The serologic specificity of I.C was mainly feline respiratory viruses and in some FeLV positive cats against the FOCMA-antigen.

Significance to Cancer Research: Progressive cases of feline leukemia have lower levels of circulating immune-complexes than regressor or carrier animals. This may be due to virus-induced immune-depression. Immune-complexes unrelated to feline leukemia were frequently encountered.

Project Officer: Ms. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human PBLs

Principal Investigator:	Dr. Nathan Sharon
Name/Address	Weizmann Institute
Performing Organization:	Rehovot, Israel

Contract Number: N01-CB-74163

Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: Develop new methods for isolating and characterizing human peripheral blood lymphocytes.

Approach: Develop procedures for cell fractionation by selective agglutination with lectins. Mononuclear cells from both healthy donors and cancer patients will be screened for their ability to bind fluorescent derivatives of peanut, soybean and wheat germ agglutinins.

Progress: We have now used agglutination with peanut agglutinin (PNA) for the separation of normal human thymocytes into two subpopulations. The PNA positive thymocytes did not respond to the mitogenic stimulation of phytohemagglutinin, nor did they respond in the mixed lymphocyte culture test, whereas the PNA negative subpopulation responded to both stimuli. It seems, therefore, that PNA positive human thymocytes are functionnally immature. In the mouse this subpopulation is sensitive to hydrocortisone treatment and can be selectively eliminated. PNA provides a unique tool for the separation of mature from immature human thymocytes. The PBL of 20 normal donors were tested with fluorescein-labeled PNA, and it was found that less than 2% of lymphocytes bind the lectin. Pathologic cells of different types of leukemia vary in their PNA binding properties. Most acute lymphatic leukemia (7/11), stem cell leukemia (7/9) and myeloid leukemia (5/7) blasts were PNA positive. Four cases of Burkitt tumor and one case of IgG positive acute leukemia were also found to be PNA positive. In contrast, the lymphocytes of the majority of chronic lymphatic leukemia patients tested (32) did not bind PNA. However, a subpopulation of PNA positive lymphocytes were found in three of the patients, all of which had low and stable white cell counts.

Significance to Cancer Research: The presence of PNA receptors or the blastic cells of patients with acute leukemia could be of therapeutic importance, since they may serve as selective targets for PNA-bound drugs.

Project Officer: Ms. Judith M. Whalen

Program: Tumor Immunology Program

Technical Review Group: Committee on Cancer Immunodiagnosis

FY 78 Funds: $86,513

Title: Search for New Human Tumor-associated Antigens in Carcinoma of the Lung

Principal Investigator:	Dr. Robert W. Veltri
Name/Address	West Virginia University
Performing Organization:	Morgantown, WV

Contract Number: N01-CB-43890
Starting Date: 5/16/74 Expiration Date: 5/15/78

Goal: Search for new tumor-associated antigens in carcinoma of the lung, which may be more useful in immunodiagnosis than currently available markers.

Approach: Lung tumor tissue, normal lungs, and embryonic lungs are processed by a variety of chemical, physical, and mechanical procedures. Antisera to these soluble and membrane extracts are raised in rabbits and absorbed until reactive only with tumor extracts. The antigens thus identified are further characterized and purified for the development of sensitive serum screening procedures.

Progress: Using the above approach, three proteins have been identified in soluble extracts of human lung carcinomas. These have been designated LTAA 1,2,3 and found to be normal proteins present in large excess in these extracts. LTAA 2 and 3 have been identified as ferritin and lactoferrin respectively. Two other proteins designated LTAA 4 and 5 have been identified in Triton X-100 extracts of lung tumor membranes. As was the case for the soluble markers, the membrane-associated LTAA 4 and 5 are not CEA, AFP or β-2 microglobulin.

Emphasis in the past year has been placed on the development of a radioimmunoassay to test the value of detecting LTAA 4 or an antibody to LTAA-4 in the serum of lung cancer patients. LTAA-4 has been purified by a combination of DEAE-cellulose chromatography, Sephadex G-200 gel filtration and isoelectric focusing. Goat antiserum to LTAA-4 has been raised and the RIA is in development. Further testing has shown that LTAA-4 is also produced by lung tumor cell lines as well as primary tumors and can be detected by indirect immunofluorescence. Preliminary results also indicate that immunocompetent lung cancer patients give a positive skin test reaction in 48 hours to LTAA-4.

Significance to Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: J. Frederick Mushinski, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Intrapleural BCG After Primary Surgery for Lung Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Martin F. McKneally Albany Medical College Albany, NY

Contract Number: N01-CB-53940
Starting Date: 6/1/75 Expiration Date: 9/30/78

Goal: To conduct preclinical and clinical evaluation of the therapeutic efficacy of intrapleural BCG with or without tumor antigen preparations in Stage I, II and III lung cancer.

Approach: Following surgical resection of their tumors, patients with lung cancer had been randomly assigned to receive intrapleural BCG plus INH or INH alone. Subsequently, the design was altered so that test Stage I patients now receive BCG plus INH followed by additional supplemental doses of BCG, while control patients received the BCG plus INH but no supplement. Stage III test patients now receive BCG + INH + supplemental BCG; the control patients receive no immunotherapy after surgery.

Progress: Stage I lung cancer patients treated with intrapleural BCG and INH continued to show a decreased incidence of tumor recurrence when compared to patients treated with INH alone. Similarly, the two groups of Stage I patients treated with intrapleural BCG and INH in the next phase of the study showed a reduced incidence of recurrent cancer when compared to the earlier randomized control group and historical controls in this institution.

Significance to Cancer Research: This randomized control trial shows a statistically significant treatment effect due to immunotherapy. A repeat trial with larger numbers of patients will be required to confirm the result.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $120,972

CONTRACT RESEARCH SUMMARY

Title: Thyroiditis as Immunotherapy

Principal Investigator: Name/Address Performing Organization:	Dr. Paul LoGerfo Columbia University New York, NY

Contract Number: N01-CB-64048
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: The purpose of this contract is to develop a form of immunotherapy for thyroid cancer.

Approach: Autoimmunity to thyroglobulin is being used to induce thyroiditis in animals by using altered forms of homologous thyroglobulin. The eventual plan is to destroy thyroglobulin producing cancers by inducing autoimmunity to thyroglobulin.

Progress: During the past year we have studied various haptenic agents for their effectiveness in producing a form of thyroglobulin which would be most effective in inducing thyroiditis. These haptens included succinic anhydride, dinitrochlorobenzene, arsinilic and sulfanilic acids. A combination of the haptens arsinilic and sulfanilic acids was clearly more effective than any of the others in producing thyroiditis when combined with Freund's adjuvant. We were able to induce autoantibodies in 95% of the rabbits and thyroiditis in 70% compared to 16% and 33% in rats. For reasons which are not clear, the rabbit provided a better model than the rat for induction of thyroditis. After a large number of studies in rats we have switched to rabbits as our animal model. No adverse side effects were noted in these animals except for some mild hypothyroidism. Long term studies with rabbits are now in progress. A protocol to treat humans with advanced thyroid cancer was submitted and approved.

Significance to Cancer Research: This is a form of specific immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Long-Term Tumor-Specific Cytotoxic Lymphocytes in Treatment of Mouse Leukemia

Principal Investigator:	Dr. Kendall A. Smith
Name/Address	Dartmouth Medical School
Performing Organization:	Hanover, NH

Contract Number: N01-CB-74141
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To evaluate the usefulness of tumor-specific cytotoxic lymphocytes maintained in continuous culture for the prevention and treatment of mouse leukemia.

Approach: Attempts will be made to (1) demonstrate the tumor-antigen reactivity of human and murine cytotoxic T-cell lines; (2) test in vivo the immunoprophylactic and immunotherapeutic value of murine cytotoxic T-cells maintained in continuous proliferative culture; and (3) to characterize and purify the growth factor which allows for the long-term culture of cytotoxic lymphocytes.

Progress: T-cells have been shown to become cytolytic to tumor cells in vitro after mixed tumor-lymphocyte culture. This activity correlates with an inhibitory effect on in vivo tumor growth. We have described a method (Nature 268:154, 1977) which allows for the maintenance of differentiated tumor-specific cytotoxic T-cells in continuous exponential proliferative culture and have demonstrated that such T-cell lines are effective in vivo in retarding tumor growth. We have continuously cultured cytotoxic T-cells for more than one year and have found that these cells maintain their morphologic and functional characteristics. The maintenance of these cells is dependent upon a T-cell growth factor (TCGF) which is produced by T-cell mitogen or antigen stimulated lymphocytes. The TCGF is consumed by proliferating T-cells and is obligatory for their continued growth.

Significance to Cancer Research: The ability to cultivate large quantities of tumor-specific cytotoxic lymphocytes which retard in vivo tumor growth may provide a new approach to cancer treatment. The successful culture of differentiated functional T-cells for extended periods offers the promise of new understanding of the mechanisms of cellular immunity and of immunodeficient and hyperimmune disease states.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $98,500

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator:	Dr. John R. McLaren
Name/Address	Emory University
Performing Organization:	Atlanta, GA

Contract Number: N01-CB-64061

Starting Date: 7/16/76 Expiration Date: 6/15/79

Goal: To determine whether or not radiation therapy modifies the immunocompetence of patients with lung, head and neck, or cervical malignancies and to determine whether or not aggressive immunotherapy with combined BCG and levamisole may alter such modifications in immunocompetence and correlate with disease-free intervals or survival times.

Approach: T- and B-lymphocyte levels and percentages, lymphocyte cytotoxicity, circulating immune complexes, *in vitro* lymphocyte transformation by mitogen, and sialic acid determinations are made on cancer patients at appropriate intervals before and following radiation therapy, and on a control population. Following radiation therapy, patients are randomized into arms with or without immunotherapy. Clinical, laboratory, X-ray, and immunological evaluations will be continued for sufficient intervals to observe disease-free intervals and survival times.

Progress: Patients with head and neck cancer, lung cancer, and cervical cancer have significantly lower than normal values on most of the above tests when evaluated prior to treatment. Following radiation therapy, these values decrease further. Following radiation therapy half the patients receive no further treatment and half are treated with immunotherapy. Both groups are evaluated at 8 and 16 weeks. Preliminary data indicate that immunocompetence rises gradually. There is some suggestion that recovery is less rapid in the immunotherapy treated group.

Significance to Cancer Research: The data corroborate mounting evidence that cancer patients have suppressed immunocompetence, and that radiation further depresses many of these immune parameters. It is important to learn the significance of depression upon relapse and survival times.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $130,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigators Name/Address Performing Organization:	Dr. Karl Erik Hellstrom Fred Hutchinson Cancer Research Center Seattle, WA

Contract Number: N01-CB-64018
Starting Date: 10/30/74 Expiration date: 3/31/78

Goal: To evaluate the clinical effectiveness of BCG scarification plus unblocking plasma versus BCG scarification plus normal plasma in patients with stage II or III malignant melanoma.

Approach: Our approach to immunotherapy is based on the demonstration of "blocking" factors present in the sera of animals and humans with progressive neoplastic disease. These blocking factors can abrogate the cytotoxic effects of host lymphocytes immune to tumor target cells in vitro. It has been proposed that serum blocking factors may play a role in tumor progression. The demonstration of "unblocking" factors present in the sera of animals or patients who are tumor-free has lent additional support to this concept and suggested that the passive administration of plasma or serum with unblocking activity may be of potential therapeutic benefit. Therefore, plasma known to contain unblocking factors has been given to patients with melanoma in stages II and III. The patients have also received BCG scarfication.

Progress: The effect of administration of "unblocking" plasma from normal Black donors was compared to that of normal Caucasian plasma in patients with malignant melanoma. Stage II and III patients with no clinically evident disease at randomization received plasma and BCG scarfication on alternate weeks. Stage III patients with nonresectable disease received the same immunotherapy, combined with systemic chemotherapy (DTIC). A conventional nonimmunotherapy control group was included in the first year of the study. One hundred and eight evaluable patients (stage II-58, stage III-50) were entered on the protocol. No difference in progression rate or survival has been observed in patients receiving unblocking plasma compared to control plasma. Similarly, no differences have been observed when patients receiving immunotherapy have been compared with patients in the nonimmunotherapy control group.

Significance to Cancer Research: This study represents the evaluation of a mode of immunotherapy previously regarded as having potential promise in cancer therapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 funds: $77,413

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Outbred Cat Lymphomas and Leukemias

Principal Investigator: Name/Address Performing Organization:	Dr. Myron Essex Harvard College Cambridge, MA

Contract Number: N01-CB-64001
Starting Date: 6/1/76 Expiration Date: 5/31/79

Goal: To see if immunotherapy using antibody to a specific tumor antigen can induce and/or sustain clinical remission in outbred cats with naturally occurring leukemia and lymphoma.

Approach: We will use specific immunotherapy, directed to the feline oncornavirus-associated cell membrane antigen (FOCMA), with and without chemotherapy. Cats with induced and naturally occurring thymic lymphoma and acute lymphoblastic leukemia will be treated with both passive and active immunotherapy.

Progress: Lymphoid leukemia cells from cats with either naturally occurring or induced leukemia possess specific neoantigens (FOCMA) on their membranes. FOCMA does not cross-react with the feline leukemia virus (FeLV) structural proteins. Serum antibody levels to FOCMA are inversely correlated with tumor development and progression. This response presumably represents a natural immunosurveillance mechanism. Large pools of feline antibody to FOCMA were developed and characterized. Antiserum for therapy was titered on both the established feline lymphoma FeLV producer line and on the FOCMA positive FeLV negative mink lines. Lysis of FOCMA containing lymphoma cells was demonstrated *in vitro* using the ^{51}Cr test. Thus far, regressions were induced in 3 of 5 cats with spontaneous lymphoma.

Significance to Cancer Research: The cat system is probably the only model where studies with spontaneous lymphoid tumors are both economically and scientifically feasible because sufficient knowledge of the tumor antigen-immune system has been developed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $85,450

CONTRACT RESEARCH SUMMARY

Title: Adjuvant Tumor-Specific Active Immunotherapy of Squamous Cell Carcinoma of the Lung

Principal Investigator:	Dr. Hiroshi Takita
Name/Address	Health Research, Inc.
Performing Organization:	Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-64007
Starting Date: 6/16/76 Expiration Date: 6/15/79

Goal: To evaluate the therapeutic effects of immunotherapy with tumor vaccine given adjuvant to curative surgical resection in squamous cell carcinoma of the lung by a prospective randomized study.

Approach: Following lung resection, patients with Stage I and II squamous cell lung carcinoma are randomized into 3 groups: Group 1-control (no treatment); Group 2-specific immunotherapy; and Group 3-nonspecific immunotherapy. The specific immunotherapy group will receive solubilized squamous cell lung carcinoma antigen (500 mcg) with complete Freund's adjuvant. The nonspecific immunotherapy group will receive Freund's adjuvant only.

Progress: A total of 41 patients with Stage I and II squamous cell lung carcinoma following curative lung resection were randomized into 3 arm study: Group 1-control; Group 2-tumor vaccine; and Group 3-Fruend's adjuvant. Two of the 13 patients in Group 1 are dead of recurrence, one of the 13 patients in group 3 is dead of recurrence, while all of the 15 patients in Group 2 are alive without disease.

Skin tests with the squamous cell lung carcinoma antigen, membrane immunofluorescence antibody assay using cultured lung tumor cells as target, and in vitro lymphocyte stimulation test showed higher values in the patients who received specific immunotherapy, indicating effective sensitization of the immunized patients by the tumor antigen.

Significance to Cancer Research: The interim results of our study indicate that adjuvant tumor specific immunotherapy may be effective. Immunologic monitoring of the patients reveals possible effective sensitization of the immunized patients to the tumor antigen.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $123,000

CONTRACT RESEARCH SUMMARY

Title: Adoptive Immunotherapy of Murine Leukemias Using Lymphoid Cells Sensitized *In Vitro* to Leukemia Antigens

Principal Investigator: Dr. Eliezer Kedar
Name/Address Hebrew University
Performing Organization: Jerusalem, Israel

Contract Number: N01-CB-64050
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To develop an experimental model for the immunotherapy of leukemia (and possibly of other malignancies) using conventional therapeutic modalities in conjunction with adoptive transfer of lymphoid cells sensitized *in vitro* to tumor cells.

Approach: Lymphoid cells from normal and tumor-bearing mice are stimulated by inactivated leukemic cells under carefully defined tissue culture conditions. The sensitized lymphoid cells are then assessed for their capacity to specifically destroy tumor cells *in vitro* and to eradicate leukemia implants *in vivo*.

Progress: (1) Improved tissue culture techniques have been established to generate large numbers of anti-leukemia cytotoxic cells. (2) The induction *in vitro* of cytotoxic reactivity to poorly immunogenic syngeneic tumor cells has been markedly amplified by employing in the cultures chemically or enzymatically modified tumor cells and immunopotentiating agents. (3) The *in vitro* generated sensitized cells have been shown to specifically prevent leukemia development *in vivo*. (4) A high proportion (over 60%) of mice bearing lethal leukemic isografts were cured using combined treatment of chemotherapeutic drugs and cytotoxic lymphoid cells sensitized *in vitro*. (5) Suppressor cells induced in the sensitization cultures have been characterized, and means have been developed for their elimination. (6) Lymphocytes cryopreserved for several months either before or after *in vitro* sensitization maintain their full capacity to react with tumor cells *in vitro* and *in vivo*.

Significance to Cancer Research: This study has already demonstrated the efficacy of *in vitro* sensitized lymphoid cells in impeding tumor growth *in vivo*. It is expected that this approach could also be applied to the treatment of human malignancies.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $98,844

CONTRACT RESEARCH SUMMARY

Title: Preparation and Distribution of Rabbit Serum Complement

Principal Investigator:	Dr. Marianna Cherry
Name/Address	Jackson Laboratory
Performing Organization:	Bar Harbor, ME

Contract Number: N01-CB-53870
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To determine whether any inbred strains of rabbits produce a serum with a consistently high level of serum complement and, if so, to investigate ways of making this reagent available to the research community for testing normal and malignant cells.

Approach: To screen sera from 10 rabbits of each sex from each of 10 inbred or partially inbred rabbit strains in our colony by complement-dependent cytotoxic assays, first with mouse target cells (normal and malignant) and then, in cooperation with other laboratories, with human cells of normal or malignant origin.

Progress: Complement-dependent cytotoxic assays are widely used in studies of human cells, both normal and malignant, for identification of antigens of the cell membrane, particularly those specific for malignant cells. Complement, a collective group of serum proteins, is an absolutely requisite reagent for such tests, and must be both high in specific activity and low in nonspecific toxicity for the cells to be tested. Among random-bred rabbits, the species of choice, only 1 animal in 40 produces acceptable complement by these criteria. By tests with normal and malignant cells from mice, it was shown during the first full year of this contract that certain inbred strains of rabbits consistently produce better complement than others. Preparations are now under way for sending selected samples of rabbit serum to several outside investigators for complement assays on human cells.

Significance to Cancer Research: Alteration of cell membrane antigens generally accompanies transformation from normal to neoplastic cells. Studies of these antigens are difficult technically, the more so due to the lack of a reliable source of complement. If certain inbred rabbit strains do, as we believe, provide high specific complement levels, the many ongoing projects in this area should be materially assisted.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: In Vitro Immunization with Autochthonous Human Tumor

Principal Investigator:	Dr. Eva Klein
Name/Address	Karolinska Institute
Performing Organization:	Stockholm, Sweden

Contract Number: N01-CB-74144
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To demonstrate the existence of specific human tumor-associated antigens and to show *in vitro* increased responses to such antigens after *in vitro* immunization with autochthonous tumor.

Approach: Human tumor specimens (carcinoma of the lung, astrocytomas, osteo- and soft tissue sarcomas) will be characterized and fractionated for tumor and lymphoid cells. Cytotoxicity tests will be performed with biopsy target cells using blood lymphocytes and tumor infiltrating lymphocytes, as effectors, both directly at harvest and after cocultivation *in vitro*. Attempts will be made to characterize the nature of the effector cell.

Progress: Separation techniques were worked out to fractionate lung carcinoma and osteosarcoma biopsies. The majority of the tumor infiltrating lymphocytes were T-cells. Purified T-cells of breast carcinoma patients were noncytotoxic against allogeneic breast carcinoma lines. An autologous test was worked out, using biopsy targets. Lymph node cells, peripheral lymphocytes, and tumor infiltrating lymphocytes in certain patients were cytotoxic. The cytotoxicity of killer T-cells could be increased by autologous sensitization. In some cases, noncytotoxic T-cells became cytotoxic following sensitization of the autologous biopsy. Killing was specific for the sensitizing autologous target. In parallel tests, autologous T-cell stimulation by the tumor biopsy (ATS) occurred more frequently than ALC (autologous lymphocytotoxicity against the same target). Using biopsy targets, NK- (natural killer) effects are minimal or nonexisting.

Significance to Cancer Research: The question whether the human tumor patient reacts against and/or can be sensitized against his autologous tumor is the central problem of human tumor immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $67,000

Title: Test Device with Standardized Antigens to Assay Delayed Hypersensitivity

Principal Investigator: Name/Address Performing Organization:	Gary L. Hein Lincoln Laboratories, Inc. Decatur, IL

Contract Number: N01-CB-43954
Starting Date: 6/30/74 Expiration Date: 7/31/78

Goal: To develop standardized "recall" antigens and a multi-test device to be used in studying skin test responsiveness in patients receiving immunotherapy.

Approach: Standardized bacterial and fungal antigens have been developed in a concentrated form which will permit their use on a multipuncture skin test device.

Progress: The device which simultaneously administers eight different antigens has been completed, and the following antigens have been developed: Streptococcus, Candida, Trichophyton, Proteus, diphtheria toxoid, tetanus toxoid and tuberculin, old. The optimal test dose for each antigen, that is, the minimal dose needed to cause a significant delayed hypersensitivity skin test reaction in a maximum number of healthy individuals, has been established in more than 1,000 human volunteers. Reaction rates and sizes have been recorded by age group and sex for each antigen. Duplicate test sites using the same antigen were compared in cancer patients and showed good reproducibility of results. Data from healthy subjects and cancer patients show that extent of induration is dose related, and that significant reductions in reactor rates and sizes occur at 1:3 and 1:10 dilutions of optimal dose.

Significance to Cancer Research: By providing cancer researchers with a battery of standardized skin test antigens and a testing device that allows uniform administration and reproducible results, it is hoped that more accurate and convenient assessment of cell-mediated immunity will be possible, aiding in diagnosis, prognosis, and selection of treatment.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $3,822

CONTRACT RESEARCH SUMMARY

Title: Squamous Cell Carcinoma of the Lung Treated by Resection or Radiotherapy

Principal Investigator: Dr. Engracio P. Cortes
Name/Address Long Island Jewish-Hillside Medical Center
Performing Organization: New Hyde Park, NY

Contract Number: N01-CB-64064
Starting Date: 6/28/76
Expiration Date: 6/27/78

Goal: To determine the efficacy of MER in Stages I and II squamous cell carcinoma of the lung (SCC) treated by resection and/or radiotherapy and to determine the efficacy of MER vs. vincristine + adriamycin + cyclophosphamide (VAC) vs. MER + VAC in Stage III SCC treated with radiotherapy with or without prophylactic brain irradiation.

Approach: Randomization is done according to stage stratification:

Stage I (completely resected)
    Arm I - No MER
    Arm II - MER
Stage II (completely resected)
    Arm I - No MER
    Arm II - MER

Stage III (RT)
    Arm I - MER)
    Arm II - VAC) ± Brain RT
    Arm III - MER + VAC)

Progress: Sixteen patients who had undergone surgery (11 Stage I and 5 Stage II) and radiotherapy (Stage II only) for SCC of the lung were randomized to receive the following treatment arms: Arm I (No MER), 8 patients; Arm II (MER), 8 patients. All (11) patients in Stage I on either arm remained free of disease from 1+ to 18+ mos. follow-up. Of 5 patients in Stage II, 2 of 3 relapsed on days 43 and 120 after randomization to NO MER and 1 of 2 on day 292 after MER therapy. Twenty-two patients with Stage III SCC of the lung, previously treated with radiotherapy, were randomized into 3 treatment arms with or without prophylactic brain irradiation. The results are:

	ARM	No. Pts.	Objective Response	Mean Survival (mos.)	Range
I-	MER	9	0	6.9	0.5-17+
II-	VAC	6	2	4.2	1.5-11+
III-	MER + VAC	7	3	6.5	1.0-16+

Each of the above treatment arms was given every 4 weeks for 12 courses. The administration of either MER, VAC or MER + VAC is tolerable.

Significance to Cancer Research: The study is ongoing and it is too early to draw conclusions.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Responses

Principal Investigator:	Dr. Michael J. O'Connell
Name/Address	Mayo Foundation
Performing Organization:	Rochester, MN

Contract Number: N01-CB-53874
Starting Date: 6/1/75 Expiration Date: 11/31/78

Goal: To evaluate the effects on immunologic determinants, therapeutic effects, and toxicity of BCG-Tice strain (lyophilized) versus BCG-Pasteur strain (lyophilized) versus BCG-Pasteur strain (fresh frozen) in patients with advanced cancer.

Approach: Patients, stratified by performance status and reactivity to recall antigen skin tests, are randomized on a double blind basis to one of the three BCG-preparations or to a placebo. Treatments are given by the tine technique on either weekly or monthly schedules, depending upon distance from the Mayo Foundation. Serial assessments of several immune parameters are made.

Progress: As of November 1, 1977, 113 patients had entered the clinical protocol. No serious toxic reactions to BCG treatments have been observed. The BCG Pasteur fresh frozen treatment regimen was closed to patient entry in June of 1977 because of unavailability of this material for clinical trial. There has been no evidence of objective tumor regressions in patients with measurable metastatic lesions resulting from the BCG treatments. Likewise, there has been no difference in the frequency of improved performance status or symptomatic status in patients receiving BCG as opposed to placebo. Inadequate numbers of patients have been treated on the weekly regimen to allow meaningful comparisons of the weekly versus monthly treatment schedules at this time.

Significance to Cancer Research: The value of using the patient with advanced refractory cancer as a model for the study of immune stimulants will be determined. Identification of a more potent and/or less toxic form of BCG preparation would have practical clinical applications to future clinical trials involving BCG immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $39,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Disseminated Human Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Evan Hersh M. D. Anderson Hospital and Tumor Institute University of Texas System Cancer Center Houston, TX

Contract Number: N01-CB-33888
Starting Date: 10/25/74 Expiration Date: 5/24/79

Goal: To develop immunological treatments for patients with human cancer.

Approach: Patients receive phase I and phase II immunotherapy agents including BCG, *C. parvum*, MER, *Pseudomonas* vaccine, Levamisole, Thymosin, and purified tumor antigen. These fall into the approaches of active-nonspecific, active-specific and immunorestorative immunotherapy. Patients are treated either after surgery when they have no evident disease, or in combination with chemotherapy for potential or actual metastatic disease.

Progress: Data evaluated during the last year has indicated that the administration of BCG by scarification to patients with stage I malignant melanoma prolongs the disease-free interval and survival. Continued follow-up of patients in our earlier BCG and DTIC + BCG immunotherapy studies for stage III melanoma, shows that there is a significant effect on remission, duration and survival in patients with less that 5 nodes, but a marginal effect on patients with greater than 5 nodes. Administration of *C. parvum* by daily intravenous infusion for 14 days repeated every 3 months combined with DTIC + Actinomycin-D chemotherapy has resulted in excellent results in patients with metastatic melanoma (stage IV). The overall response rate is approximately 38% and the median response duration in patients under the age of 60 is in excess of 1 year. In stage II breast cancer, more than 150 patients have now been entered into a program of FU, Adriamycin, Cytoxan, and BCG immunotherapy, and there is a significant prolongation of disease-free interval and survival. In earlier studies therapeutic results were compared to historical controls. Newer phase III studies are prospectively randomized.

Significance to Cancer Research: Immunotherapy, using a variety of agents has been shown to be effective in remission and survival prolongation in a number of human malignancies.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $222,475

Title: Immunotherapy in the L_2C Guinea Pig Leukemia

Principal Investigator: Name/Address Performing Organization:	Dr. Samuel G. Murphy M.D. Anderson Hospital and Tumor Institute University of Texas System Cancer Center Houston, TX

Contract Number: N01-CB-64003
Starting Date: 6/30/76 Expiration Date: 6/30/78

Goal: To evaluate dose, route, and schedule of administration of immunotherapeutic agents and to investigate the structuring of sequential and combination immunotherapeutic agents in the L_2C guinea pig leukemia model.

Approach: Animals with leukemia are treated with systemic chemotherapy and subsequent radiation therapy to the cranial spinal axis to prevent central nervous system leukemia. During the period of complete remission, animals are randomized to experimental groups and treated with immontherapeutic agents. Two variables such as route and schedule of administration are held constant and dosage is varied in the experimental groups. Animals are monitored by peripheral blood counts to determine the duration of remission.

Progress: Preliminary studies with Corynebacterium parvum had suggested that this agent had therapeutic potential when administered intraperitoneally in a multiple dose schedule. However, confirmatory experiments failed to support this favorable conclusion. Studies are in progress using BCG. Investigation will be made of schedule dependency for administration by the intracardiac and intradermal routes.

Significance to Cancer Research: This animal model was developed to investigate the interaction of specific and nonspecific immunotherapy in an animal model whose pathophysiology and therapy of disease closely resembled human acute leukemia. This model should be helpful in establishing biological variables that influence the effectiveness of immunotherapeutic agents.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Name/Address Performing Organization:	Dr. Gerald L. Bartlett Milton S. Hershey Medical Center Hershey, PA

Contract Number: N01-CB-33891
Starting Date: 6/30/73 Expiration Date: 10/31/78

Goal: To develop animal tumor models which will be useful for evaluating immune stimulants as immunotherapeutic agents.

Approach: In four different tumor-host models, animals are given immunotherapy at various times after tumor implantation, either alone or in combination with other means of treatment. The effect of the immunotherapy and the role of immune stimulants in immunotherapy are evaluated in terms of improved survival of treated animals. Animals which survive are tested further for improved tumor rejection immunity.

Progress: Studies were carried out using 1) line 10 hepatoma in Strain 2 guinea pigs, 2) LSTRA mouse leukemia, 3) 13762 A rat mammary carcinoma and 4) CaD2 mouse mammary carcinoma. Tests were variously performed with cultured tumor cells, lyophilized tumor cells in suspension or in oil droplets, C. parvum, BCG cell walls in oil droplets, BCG cell wall skeletons + P3 in oil droplets and seven BCG preparations. There were some favorable results. Yet, the results were highly variable and emphasized the uncertainty of reliance on any single assay for the evaluation of immune treatments or of immune stimulants, and they further emphasized the hazard of drawing "generalizations" from results in a single experimental or clinical setting.

Significance to Cancer Research: These studies demonstrate that caution must be exercised in drawing generalizations on immunotherapy from a single experimental or clinical result.

Program Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $151,373

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: Name/Address Performing Organization:	Dr. James F. Holland Mt. Sinai School of Medicine New York, NY

Contract Number: N01-CB-43879
Starting Date: 6/30/74 Expiration Date: 3/20/79

Goal: To evaluate the usefulness of neuraminidase-treated allogeneic AML cells (VCN-AML) in acute myelocytic leukemia (AML).

Approach: Following remission induction with cytosine, arabinoside, and daunorubicin, AML patients had earlier been randomly assigned to maintenance chemotherapy alone, chemotherapy in combination with VCN-AML or in combination with VCN-AML plus MER. The MER arm has been deleted from the protocol. Patients are studied with both _in vivo_ and _in vitro_ immunologic tests.

Progress: Of the 102 patients entered for study, the remission duration for the 32 patients receiving chemotherapy only was 42 weeks; for those receiving chemotherapy plus VCN-AML plus MER, the remission duration was 53 weeks; while for the 45 patients who received chemotherapy plus VCN-AML the calculated remission duration is 126 weeks. These preliminary data showing improvement to 126 weeks represent a highly significant difference from the remission duration of the other groups. _In vivo_ and _in vitro_ immunodiagnostic tests indicate the restoration of normal immunocompetence.

Significance to Cancer Research: Preliminary data analysis suggests that the combination of sustaining chemotherapy with neuraminidase-treated allogeneic AML cells may represent a significant advance in the treatment of AML.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $139,621

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Fibrosarcomas of Chickens

Principal Investigator: Name/Address Performing Organization:	Dr. Stephen P. Lerman New York University Medical Center New York, NY

Contract Number: N01-CB-64043
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the usefulness of various immunotherapeutic approaches in transplantable and primary carcinogen-induced fibrosarcomas in histocompatible SC strain chickens.

Approach: Methods of inducing specific immunity to individual fibrosarcoma lines in the same strain will be developed optimally with respect to sites, dose, and frequency of injections. Particular attention will be given to the use of C. parvum (CP).

Progress: The i.v. injection of 10^9 CP cells into chickens bearing progressively growing tumors followed by a series of 6 intratumor injections of 10^7 CP over a 2-1/2 month period failed to significantly alter the distribution of progressive vs. regressive tumors in comparison to that found in controls not injected with CP. An i.v. injection of 10^9 CP into bearers of progressively growing tumors followed one month later by 10^7 CP i.v., 3 hrs after exposure of tumors to 450R local x-irradiation, also failed to result in tumor regression. As another approach, tumor bearers were injected with 10^9 CP followed 1-3 wks later by excision of a large portion of the tumor mass. Cells or pieces of the autochthonous tumor were then injected alone in one wing and with 10^7 CP into the other wing. At the same time, most tumors were established in culture. In most cases, tumors grew progressively in the wing injected with tumor alone while the primary tumor continued to increase in size. To understand better these failures to induce immunity, our efforts are now devoted to demonstrating the immunogenicity of primary tumors upon transplantation to other animals with and without CP, and to show concomitant immunity in the primary host.

Significance to Cancer Research: The information to be gained is essential to development of immunotherapeutic procedures.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Quantitative Assays of Monocyte-Macrophage Function

Principal Investigator:	Dr. Gerald W. King
Name/Address	Ohio State University
Performing Organization:	Columbus, OH

Contract Number: N01-CB-53936
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To develop quantitative in vitro assays of human monocyte-macrophage function that will reflect in vivo functional status and define further the role of this cell system in immune responses.

Approach: To develop and evaluate for reproducibility various assays of blood monocyte function using normal cells. These assays are then performed on patients with resected malignant melanoma who are receiving adjuvant immunotherapy or patients with disseminated tuberculosis or cancer. This defines the efficacy of the assays in situations of functional stimulation or suppression.

Progress: Several new assays of monocyte target cell interaction have been established. These include direct monocyte cytotoxicity toward several tumor cell lines including HeLa and HCT colon carcinoma. These studies demonstrate clearly that normal human monocytes are capable of producing cytotoxicity as determined by release of radioactive thymidine from thymidine labeled tumor cells over a 48-96 hour period. A second major development has been the first clear delineation of human monocyte antibody dependent cytotoxicity directed toward nucleated malignant cell lines. This has been demonstrated in two independent systems, the first utilizing CEM lymphoblasts as the target cell and HLA antibodies from human donors (multiply transfused) as the antibody source.

Significance to Cancer Research: The development of in vitro assays for in vivo immunologic status represents an important element in the ultimate development of effective immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New Approaches to Immunotherapy

Principal Investigator:	Dr. Jerald J. Killion
Name/Address	Oklahoma Medical Research Foundation
Performing Organization:	Oklahoma City, OK

Contract Number: N01-CB-53937
Starting Date: 6/27/75 Expiration Date: 9/26/77

Goal: To improve the use of active, specific immunotherapy of cancer by studying the immunotherapeutic value of isolated tumor cell subpopulations.

Approach: Murine tumor cell subpopulations are isolated by cell-affinity chromatography from lectin-nylon columns. This procedure separates tumor cells based upon their expression of cell-surface carbohydrates. These subpopulations are then used in immunotherapy protocols of L1210 leukemia (following reduction in tumor burden by chemotherapy).

Progress: The studies have shown the following general trends in combination chemotherapy-immunotherapy of L1210 leukemia: (a) cells isolated from either concanavalin A-columns or fucose protein-columns were effective in immunotherapy; and (b) cells isolated from galactosamine-specific columns were deleterious, i.e., the rate of death of mice treated with these subpopulations was faster than those mice treated with chemotherapy only. These studies have also been extended to EL4 lymphoma (combination chemotherapy-immunotherapy). Preliminary results indicate that unlike L1210 leukemia, cells isolated from galactose-specific columns are effective immunogens. Current studies are evaluating the use of tumor cell subpopulations combined with nonspecific immunostimulants, BCG and *C. parvum*.

Significance to Cancer Research: The present work indicates that specific immunotherapy may be either effective or deleterious, when combined with primary chemotherapy. Attempts to define the use of isolated tumor subpopulations in immunotherapy of residual tumor continue.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Quantitative Assays of Monocyte-Macrophage Function

Principal Investigator:	Dr. John R. David
Name/Address	Robert B. Brigham Hospital
Performing Organization:	Boston, MA

Contract Number: N01-CB-53869
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: To determine whether biochemical changes in human monocytes relate to the tumor-killing capacity of these cells.

Approach: Quantitative assays of macrophage function will be developed with guinea pig macrophages and ultimately will be applied to human macrophages. Changes in monocyte macrophage surface membranes, metabolism and tumor-killing capacity will be explored.

Progress: Purified human monocytes have been obtained from peripheral blood using Ficoll gradient centrifugation technique and adherence to monolayers. Preparations of human lymphocyte mediators (MIF and MAF) were obtained by neuraminidase-galactose oxidase treatment of lymphocytes *in vitro*. Activation of human monocytes with MIF/MAF is associated with an increase in the production of plasminogen activator as well as plasminogen independent esterases, an increase in the rate of leucine incorporation, and a rapid increase in the flux of calcium across the plasma membrane. We have also successfully been able to activate human monocytes *in vitro* by lymphocyte mediators and have shown that they will kill a number of different human tumor cell lines. We are standardizing these *in vitro* assays and plan to evaluate the function of monocytes obtained from patients with tumors.

Significance to Cancer Research: These studies should allow better evaluation of the function of human monocytes - a cell crucial in immunity to cancer - as well as provide further information on the mechanism of monocyte activation as it relates to tumor immunity.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Activated Macrophages as Immunotherapeutic Agents

Principal Investigator: Name/Address Performing Organization:	Dr. Willy F. Piessens Robert B. Brigham Hospital Boston, MA

Contract Number: N01-CB-64057
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To determine if *in vitro* activated macrophages can inhibit the growth of tumors *in vivo*.

Approach: Guinea pig macrophages are activated *in vitro* with the lymphocyte mediator, macrophage activating factor, or with endotoxin. The cells are then mixed with tumor cells and the mixture is injected subcutaneously into nonimmune animals.

Progress: Activated macrophages inhibit the local growth of the syngeneic tumors, line 1 and line 10 hepatoma, when mixed with tumor cells in the ratio of 3 macrophages per tumor cell or more. The higher this ratio, the better the therapeutic effect. So far, we have only been able to delay tumor growth but have not been able to suppress tumors completely. The inhibitory effect of syngeneic activated macrophages is less marked than that of allogeneic effector cells. Tumor growth was inhibited only when activated macrophages and tumor cells were injected at the same site. Further, none of the recipients of the activated macrophages-tumor cell mixtures developed immunity to a second tumor challenge and all ultimately died with tumors. These results clearly indicate that mediator activated macrophages can function as effector cells against tumors *in vivo* and suggest that these cells may be involved in tumor suppression following intralesional injection of unrelated antigens such as BCG.

Significance to Cancer Research: Our experiments are designed to explore a new approach to nonspecific adoptive immunotherapy using activated macrophages as immunotherapeutic agents.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens

Principal Investigator: Dr. Joseph D. Feldman
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: N01-CB-43874
Starting Date: 3/1/75 Expiration Date: 2/28/79

Goal: To evaluate the effects of immunologic agents and reagents such as antigens, antigen-antibody complexes, and effector cells on the treatment of a virus-induced rat tumor.

Approach: Attempts will be made to augment and exaggerate the host immunologic responses that inhibit or destroy tumor in vivo and to suppress those responses that enhance tumor growth. This entails preparation of (a) membrane antigens of tumor cells; (b) cytotoxic antibodies of a single immunoglobulin class and specificity; and (c) purified populations of cytotoxic effector cells.

Progress: Tumor specific proteins were isolated from surface membranes and cytosol of a BN sarcoma, MST. On slab gel electrophoresis, membrane extracts yielded 3 bands of 185,000, 150,000, and 70,000; cytosol extracts yielded 4 bands of 220,000, 170,000, 85,000, and 39,000. Both preparations specifically co-precipitated with the same anti-MST antiserum, specifically inhibited binding of anti-MST to MST, and induced antibodies in guinea pigs but not in BN rats. Antigens in circulating immune complexes of MST-bearing rats were identified as p30 and gp70; an unknown entity larger than gp70 has not yet been identified. Cytotoxic T cells from spleens of BN rats immunized with MST, MuLV-M, p30 or gp70 were infused intravenously into BN rats with 6 day established MST. T_{MST} inhibited or eliminated established MST; T_{MuLV} were less efficacious; T_{p30} were dubiously effective; T_{gp70} were ineffective. Cytotoxicity of T cells from these kinds of donors is being augmented in vitro and will be assayed against established tumor.

Significance to Cancer Research: It is essential to understand the complex interactions of the components of the immune system and to determine how best to manipulate these interactions to achieve a therapeutic benefit in cancer treatment.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $124,707

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Tumors Using Hapten-Reactive T-Lymphocytes

Principal Investigator:	Dr. David Katz
Name/Address:	Scripps Clinic and Research Foundation
Performing Organization:	La Jolla, CA

Contract Number: N01-CB-74196

Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To develop an effective method for improving host anti-tumor immunity against growth of syngeneic tumors using hapten-reactive amplifier T-cells.

Approach: Mice will be 1) pretreated with hapten-D-GL conjugates and then 2) primed with the specific hapten conjugated to mouse gamma globulin (MGG) and then 3) challenged with hapten-conjugated tumor cells. In therapeutic, as opposed to prophylactic, models, the solid tumor will be obtained by surgical extirpation or, alternatively, haptenic coupling will be attempted *in situ*.

Progress: Our previous work indicated that pretreatment with a hapten-D-GL conjugate followed by immunication with hapten-MGG generated hapten-reactive helper T cells capable of augmenting the antibody response to that hapten or the cytotoxic T cell response induced by immunization with haptenated tumor cells. Results obtained during the present contract year were: 1) Pretreatment with low-dose irradiation or cyclophosphamide (CY) augemented the net hapten reactive helper T cell activity but not to the same extent as hapten-D-GL pretreatment. Combination of irradiation or CY with hapten-D-GL pretreatment diminished that activity. 2) A survey of various mouse strains for their helper T cell reactivity to TNP revealed that all strains tested - A/J, C57BL/6, BALB/c and, to a lesser extent, C3H/He - generated TNP-reactive helper T cells. 3) The survival of TNP-MGG-primed BALB/c mice inoculated with TNP-coupled LSTRA tumor cells (of BALB/c origin) was significantly prolonged over that of primed mice that were inoculated with unmodified tumor cells. 4) In preliminary experiments, spleen cells from normal or tumor-immune mice sensitized *in vitro* to TNP-coupled tumor cells generated an augmented cell-mediated cytotoxic response in the presence of 770 R X-irradiated spleen cells primed *in vivo* to the same hapten.

Significance to Cancer Research: The use of hapten-reactive helper T cells may provide a way to augment the immune response to syngeneic tumors that are in general, weakly immunogenic, and therefore may enable us to devise better methods for the immunotherapy of cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $48,600

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Ovarian Cancer Using Specific Serotherapy in Combination with Intraperitoneal C. parvum

Principal Investigator: Name/Address Performing Organization:	Dr. Robert C. Bast Sidney Farber Cancer Center Boston, MA

Contract Number: N01-CB-74194
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To evaluate the therapeutic efficacy of specific xenogeneic serotherapy in combination with intraperitoneal C. parvum in the treatment of syngeneic murine ovarian carcinoma transplants.

Approach: Efforts will be made to determine the optimal dose, route, order, and schedule of administration for the treatment combination in mice. Attempts will be made to determine relevant in vitro correlates with disease status. Techniques will be perfected for cryopreservation of human ovarian tumor cells and for the preparation of specific xenoanti serum against human tumor cells for possible use in human clinical trials at some future date.

Progress: A combination of C. parvum and a specific xenoantiserum has been found significantly more effective than either single agent in prolonging the survival of mice that had received syngeneic ovarian carcinoma cells intraperitoneally (IP). In vitro, a combination of C. parvum activated peritoneal cells and specific xenoantiserum has also proven significantly more effective than either single component in destroying murine ovarian carcinoma cells in the absence of complement. Activation of peritoneal cells for cooperation with xenoantiserum in vitro peaked 3-7 days after a single IP injection of C. parvum and declined to baseline over 3-4 weeks. With repeated injections of C. parvum at appropriate intervals, activation of peritoneal cells could be prolonged and augmented.

Separation of murine ovarian carcinoma cells from contaminating host peritoneal cells has been accomplished using albumin gradients and a fluorescence activated cell sorter. Antisera prepared against separated tumor cells have proven sufficiently specific to permit immunoassay and affinity purification of tumor associated antigen(s).

Significance to Cancer Research: Synergy between two different forms of immunotherapy may permit more effective treatment of human cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $123,000

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Response

Principal Investigator: Name/Address Performing Organization:	Dr. Yashar Hirshaut Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-53970
Starting Date: 5/16/75 Expiration Date: 5/15/79

Goal: To evaluate selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, and effects on immune status. The agents presently being investigated are *Corynebacterium parvum* (*C. parvum*) levamisole, BCG, intravenously administered, and purified endotoxin.

Approach: Clinical protocols have been designed that provide for the administration of new immunotherapeutic agents to patients in accordance with acute, chronic, and escalating dose schedules. Where feasible, the routes of administration include oral, parenteral, and intralesional. During treatment, toxic and immune parameters are measured at regular intervals.

Progress: Phase I studies of levamisole and *C. parvum* have been completed. The MTD of levamisole was determined to be $358mg/M^2$. Toxicity is mild and related primarily to the gastrointestinal and nervous systems. The limiting toxicity proved consistently to be persistent nausea. The MTD of *C. parvum* given intravenously for 30 minutes is $12.9mg/M^2$. When infusion time is prolonged MTD increases to $128mg/M^2$. With *C. parvum*, fever and chills are the most common toxic manifestation. In addition, alterations in blood pressure and pulse occur as well as headache, nausea, vomiting, dyspnea, and cyanosis occur. Alterations in immune function have been observed after the administration of both drugs. In general, the magnitude of such changes is generally low. Most impressive have been the effects of *C. parvum* on peripheral monocyte function and lymphocyte blastogenesis in response to specific antigens.

Significance to Cancer Research: Immunotherapy is a new modality of treatment for cancer whose appropriate use depends on understanding of how available agents affect specific aspects of immune function. Phase I studies underway should assist in determining optimal doses producing acceptable toxicity.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $113,179

CONTRACT RESEARCH SUMMARY

Title: Plasmatherapy of Mouse Tumors

Primcipal Investigator: Name/Address Performing Organization:	Dr. Robert L. Kassel Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-74197
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To determine whether plasma therapy (alone or in combination with other treatment modalities, e.g., chemotherapy) will be useful in the treatment of transplantable solid tumors and in the prevention and treatment of spontaneous leukemia in mice.

Approach: Leukemic or preleukemic mice will be treated with $C5^+$ heparinized plasma or purified eluates and their survival compared with control animals treated with $C5^-$ heparinized plasma or purified $C5^-$ eluates. Plasma therapy will also be combined with chemotherapy and/or other treatment modalities. Circulating immune complexes will be assayed before, during, and after therapy.

Progress: Selective destruction of neoplastic cells has been observed in leukemic mice, cats, and dogs following infusion of normal serum, heparinized plasma, or whole blood. The antileukemia activity can be concentrated and purified by adsorption onto a calcium phosphate gel. An AKR mouse colony has been established in a bioclean room, providing the conditions for long-term experiments. Responses of leukemic mice to treatment with cytoxan have been established (remission following a single injection lasts about 10 days) and experiments involving cytoxan-plasma therapy have been initiated. Preliminary results of this combined therapy are most encouraging for we have observed a "cure" rate of approximately 18% (13/71 animals treated surviving more than 60 days with no signs of leukemia).

Significance to Cancer Research: In spite of toxic side effects, chemotherapy has been a treatment of choice because it can prolong life and in some cases "cure." Any adjunct to chemotherapy that reduces the total dose required for effective cytoreduction should reduce the toxic burden the host must bear. The synergistic action of cytoxan-plasma therapy may present a method of reducing burden and prolonging remission.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $122,500

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunotherapy with Tumor Preparation in Man

Principal Investigator:	Dr. Herbert F. Oettgen
Name/Address	Sloan-Kettering Institute for Cancer Research
Performing Organization:	New York, NY

Contract Number: N01-CB-64062
Starting Date: 6/16/76 Expiration Date: 6/15/79

Goal: To determine the effectiveness of treating patients with advanced malignant melanoma with a combination of surgery, BCG, autologous tumor cell vaccine, and chemotherapy (DTIC). To monitor the patients' immune response to surface antigens of autologous and allogeneic melanoma cells, as measured by serological and cellular reactions, and to correlate the response with the course of the disease and the effects of therapy.

Approach: Patients with malignant melanoma Stage III will be treated with BCG and a vaccine of autologous irradiated tumor cells. Patients with malignant melanoma Stage IV will receive BCG, irradiated autologous tumor cells, and DTIC. The patients' immune response to surface antigens of their own tumor cells will be tested *in vitro* by serological tests (mixed hemadsorption, immune adherence, complement-dependent cytotoxicity, antibody-directed cell-mediated cytotoxicity), and tests for lymphocyte cytotoxicity on autologous or allogeneic target cells.

Progress: From 1 July 1976 to 15 January 1977, 6 patients with malignant melanoma Stage III and 8 patients with malignant melanoma Stage IV were treated with autologous tumor cell (ATC) vaccine and BCG, alone (Stage III) or combined with DTIC (Stage IV). Serum from these patients was tested for antibodies to surface antigens of autologous cultured melanoma cells by mixed hemadsorption (MHA) and immune adherence (IA). Antibodies were detected in the serum of 4 patients by MHA, and with serum of 9 patients by IA. The specificity of antibodies in high-titered sera was defined by extensive absorption tests. Peripheral blood lymphocytes were tested for cell-mediated cytotoxicity (CMC) for autologous cultured melanoma cells prelabeled with ^{3}H-proline. CMC for autologous melanoma cells was detected in 9 of 13 patients. Correlation of the results of serological tests and lymphocyte cytotoxicity tests with vaccination and course of disease is underway. Therapeutic effects of vaccination cannot yet be evaluated.

Significance to Cancer Research: Malignant melanoma offers unique opportunities for study in that the serology is further advanced than in other types of cancer. Thus a trial of autologous vaccine will provide information concerning therapy as well as basic human cancer immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $138,000

CONTRACT RESEARCH SUMMARY

Title: Immunization with BCG and Allogeneic Renal Cancer Cells in Patients with Renal Cell Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Herbert F. Oettgen Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-74145
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To determine whether the cells of renal cancers have cross-reacting tumor-associated antigens.

Approach: Assays for humoral immunity to autologous tumor cells with be performed before and after immunization with BCG and allogeneic renal cancer cells. Attempts will be made to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics and cell cycle kinetics. Periodic assessment of humoral immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stages II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 5,6 Approach 1)

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $119,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Tumors in Mice with Tumor Necrosis Factor (TNF)

Principal Investigator:	Dr. Lloyd Old
Name/Address	Sloan-Kettering Institute for Cancer Research
Performing Organization:	New York, NY

Contract Number: N01-CB-64051
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To determine the effectiveness of tumor necrosis factor (TNF) in the therapy of transplantable and spontaneous mouse tumors.

Approach: TNF is found in the serum of BCG or C. parvum primed mice injected with endotoxin. Both TNF+ serum and partially purified TNF are being tested for antitumor activity in the following five mouse tumor systems: Meth A sarcoma (BALB/c); leukemia L1210 (DBA/2); sarcoma 19B-MCA (C57BL/6); AKR spontaneous leukemia; and C3H spontaneous mammary carcinoma. Tumor necrosis, tumor growth, inhibition of metastases, and survival are the parameters to be assessed.

Progress: The two notable features of TNF are (a) its ability to cause acute necrosis of a variety of mouse tumors growing intradermally or subcutaneously and (b) its cytostatic and cytotoxic activity on certain lines of cultured cancer cells. Standard methods for its production and partial purification are now defined. During the contract period, a large pool of TNF serum has been prepared for (a) antitumor assays and (b) production of partially purified TNF. Single injections of TNF+ serum cause acute necrosis of established Meth A and 19B-MCA solid tumors and significant reduction in the size of leukemic lymph nodes and spleen in AKR mice. Growth of leukemia L1210 was not influenced by treatment with TNF+ serum. In the Meth A and 19B-MCA systems, multiple injections of TNF+ serum were far more effective than a single injection in causing inhibition of tumor growth and tumor regression, and, in the case of 19B-MCA sarcoma, in reducing the number of pulmonary metastases.

Significance to Cancer Research: Immunological approaches to cancer therapy are being tested in laboratories and clinics around the world. Agents such as BCG and C. parvum, which augment immunological reactivity in a general fashion under certain circumstances and have tumor inhibitory activity in experimental systems, are receiving greatest attention in clinical trials of cancer immunotherapy. The past work leads to speculation that TNF may be one of the mediators responsible for the antitumor activity of these microbial immunopotentiators. Understanding how TNF causes tumor cell destruction and determining how effective it is as an antitumor agent may provide novel approaches to cancer immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $91,600

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Disseminated Carcinoma of the Breast

Principal Investigator: Name/Address Performing Organization:	Dr. Carl Pinsky Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-53873

Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: To determine whether the addition of Corynebacterium parvum to combination chemotherapy in patients with advanced breast cancer increases response rate, increases response duration or survival time, protects against the deleterious effects of chemotherapy, or augments immunologic function.

Approach: This is a prospective, randomized trial using cyclophosphamide, adriamycin, methotrexate, and 5-fluorouracil every 28 days, with or without C. parvum given weekly, subcutaneously.

Progress: By random assignment, one group of patients receives Corynebacterium parvum in addition to the chemotherapy. There have been 51 patients randomized to receive chemotherapy only, 67 to receive chemotherapy plus C. parvum. Of 45 evaluable patients receiving chemotherapy only, 24 have had a complete or partial response. Of 44 evaluable patients receiving chemoimmunotherapy, 19 have had complete or partial response. Previous results suggested that patients who responded to chemotherapy and received C. parvum lived longer than responders who received chemotherapy only. At this time, there appears to be no difference in response duration or survival. The development of leukopenia and thrombocytopenia has also been similar in the two groups. Additional toxicity has included nausea and vomiting, alopecia and stomatitis. No deaths attributable to the therapy have occurred. There are no differences in immunological reactivity in the two groups.

Significance to Cancer Research: Since complete response rate is minimal and ultimate survival continues to be short in patients with advanced breast cancer, all leads which suggest lengthened survival must be followed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $98,000

CONTRACT RESEARCH SUMMARY

Title: Intratumoral BCG Prior to Radiation and Cystectomy in Patients With Bladder Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Carl M. Pinsky Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-74142

Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To evaluate the therapeutic efficacy of intratumoral injection of BCG prior to radiation and cystectomy.

Approach: Eligible patients with solitary infiltrating bladder cancers will be randomly allocated to treatment with intratumoral injection of BCG prior to radiation and cystectomy, or to treatment with radiation and cystectomy alone. The study will evaluate the effects of BCG therapy on disease-free interval, site(s) or recurrence and survival.

Progress: New Contract. It is too early to report progress.

Significance to Cancer Research: (NCP Objective 2, 6 Approach 1, 4). More effective therapy for bladder cancer is needed. It is important to determine whether an approach that is successful in animal models will be useful in the therapy of humans.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $53,000

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy in Patients with Recurrent Superficial Bladder Cancer

Principal Investigator: Dr. Carl M. Pinsky
Name/Address Sloan-Kettering Institute for
Performing Organization: Cancer Research
New York, NY

Contract Number: N01-CB-74146
Starting Date: 9/16/77 Expiration Date: 9/15/78

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled, randomized, prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: New Contract. It is too early to report results.

Significance to Cancer Research: (NCP Objective 6 Approach 1,4). A pilot study has suggested that intravesical and percutaneous BCG decreases the number of recurrences in patients with superficial bladder carcinoma. It is important to establish that this is the case with a controlled, prospective, randomized trial.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY: 78 Funds: $47,600

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Levamisole as a Therapeutic Adjunct in Squamous Cell Carcinoma of the Head and Neck

Principal Investigator: Name/Address Performing Organization:	Dr. Elliot W. Strong Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-53875
Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: To evaluate the usefulness of levamisole in the treatment of patients with squamous cell cancer of the head and neck.

Approach: Patients stratified according to type of disease, primary or recurrent, stage and site, are randomized after surgery on a double-blind basis to receive levamisole or a placebo. Treatment is given three times on alternative weeks for two years. The effects of levamisole on clinical course as well as on immune status are evaluated.

Progress: Of the 52 patients available for study at the end of November, 1977, 26 were randomized to levamisole and 26 to placebo. There was no statistically significant difference between the proportion disease-free for the levamisole and placebo groups considering all patients or only primary disease patients. However, there were suggestive differences favoring levamisole in the recurrence rates of those patients previously untreated in Stage 2, only. Immunological testing demonstrated that DNCB responses and lymphocyte responses to mitogens were improved in patients after tumor resection prior to beginning the levamisole-placebo protocol. No significant differences in immune reactivity between the two groups was seen during therapy.

Significance to Cancer Research: Immunopotentiation is a promising clinical approach to cancer therapy especially when used as an adjunct in patients with known immunosuppression. Levamisole, an immune modulator, has been reported to be useful in the treatment of resectable lung cancer and in patients treated for locally advanced breast cancer with radiation (Rojas et al.). This study will attempt to determine whether levamisole has therapeutic efficacy in patients with head and neck cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherpay
FY 78 Funds: $96,100

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Herpes Virus Lymphomas in Marmosets

Principal Investigator: Name/Address Performing Organization:	Dr. Jorg W. Eichberg Southwest Foundation for Research and Education San Antonio, TX

Contract Number: N01-CB-64055
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: Successful immonotherapy of cancer.

Approach: The use of xenogeneic specific transfer factor (TF) and immune RNA (IRNA) in the prevention and treatment of a 100% fatal lymphoproliferative malignancy in cotton-top marmosets induced by *Herpesvirus saimiri* (HVA).

Progress: TF and/or IRNA prepared from six baboon donors were evaluated for their efficacy to transfer cellular reactivity and provide protection for HVS induced fatal lymphoma to a total of 26 cotton-top marmoset recipients. Both immunotherapeutic moieties, when administered singly or in a combined regimen, failed to prove efficacious in protecting marmosets. Besides the failure to protect from lymphoma, *in vitro* testing of lymphocytes from marmoset recipients did demonstrate only occasionally transfer of cellular reactivity by TF and/or IRNA. At no time could HVS be isolated from PBL of baboon donors, and specific serum neutralizing antibodies were low or not detectable. Cebus monkeys and common marmosets are currently being used as TF and IRNA donors. Infection of these two nonhuman primate species by HVS has been demonstrated by HVS isolation from lymphocytes.

Significance to Cancer Research: HVS lymphoproliferative disease is absolutely reproducible in cotton-top marmosets and clinically similar to human cancer. This animal model provides a direct phylogenetic relationship to man, and offers the opportunity to evaluate transfer of immune reactivity and xenogeneic immunotherapy by TF and/or IRNA.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy with In Vitro Lymphocyte Sensitization

Principal Investigator:	Dr. Henry S. Kaplan
Name/Address	Stanford University
Performing Organization:	Stanford, CA

Contract Number: N01-CB-63988
Starting Date: 9/1/75 Expiration Date: 2/28/78

Goal: (1) Development of in vitro methods for sensitization of human and mice lymphocytes against allogeneic and syngeneic tumor cells. (2) Analysis of the cell populations involved in such sensitization and of the regulatory mechanisms that control the induction and effector phases of the response. (3) Inhibition of tumor development by adoptive transfer of the in vitro sensitized lymphocytes.

Approach: Mouse spleen cells or human peripheral blood lymphocytes are sensitized in vitro against tumor cells either by direct interaction with irradiated tumor cells or by exposure to syngeneic macrophages that were fed with cell-free extracts of tumor cells. A mouse lymphoma model is used to test sensitized lymphocytes for their activity against target cells in vitro, and for their influence on tumor development in vivo.

Progress: Peripheral blood lymphocytes (PBL) from human donors were tested for natural killing (NK) activity and for two types of in vitro-induced cytotoxicity against allogeneic diffuse histiocytic lymphoma (DHL) cell lines. Culture-induced cytotoxic (CIC) activity developed in cultures of unseparated PBL incubated in the presence of fetal bovine serum (FBS), but not in the presence of human serum (HS). The CIC mediating effector cells were removed by nylon wool adherance, whereas the NK cells were not. A second type of in vitro-induced cytotoxicity was obtained by specific sensitization against DHL cells (SIC). The SIC effector cells were not retained by nylon wool. SIC was manifested following sensitization in medium containing HS but not in FBS-containing medium unless nylon wool adherent cells were removed before sensitization. The lack of SIC in the presence of FBS appeared to be due to nylon wool adherent suppressor cells. Such suppressor activity was also removed by plastic adherence or elimination of phagocytic cells. CIC and suppressive activity were found to be mediated by different cell populations.

Significance to Cancer Research: These studies helped to clarify the various types of cell-mediated immune responses and pseudo-immune responses to murine and human lymphoma cells, and may thereby facilitate the development of effective and specific in vitro sensitization against human lymphoma cells.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Modified Tumor Cell Membranes as Immunotherapeutic Agents

Principal Investigator:	Dr. Jack H. Pincus
Name/Address	Stanford Research Institute
Performing Organization:	Menlo Park, CA

Contract Number: N01-CB-64053
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To evaluate the effect of Vibrio cholerae neuraminidase (VCN) modification of plasma membranes and membrane glycoproteins on their capacity to induce an immunological regression of growing tumors. To determine whether glycosidic modifications optimizes the immunotherapeutic effectiveness of these cell-free preparations.

Approach: Purified plasma membranes are prepared from the L1210 tumor, a transplantable leukemia that is propagated in DBA/2 mice and are treated with VCN to remove membrane-bound sialic acid. The modified plasma membranes are tested for their effectiveness on inducing an immunological regression of growing L1210 tumors.

Progress: The immunogenicity of VCN-treated and untreated L1210 plasma membranes was evaluated by immunizing DBA/2 mice with these preparations and challenging them three weeks later with an intraperitoneal injection of viable L1210 cells. Only mice that received VCN-treated membranes resisted the tumor challenge. A chemo-immunotherapy model was developed to evaluate the effective membrane preparations in tumor-bearing mice consisting of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) chemotherapy and immunotherapy with either untreated or VCN-treated L1210 plasma membranes. Combination chemotherapy with untreated membranes caused enhanced tumor growth and decreased the life span. In contrast, treatment with chemotherapy combined with VCN-treated plasma membranes resulted in a longer survival than mice treated with chemotherapy only.

Significance to Cancer Research: Defining those modifications of tumor cell membranes that increase their immunogenicity, will aid in developing vaccines for use in the active immunotherapy of small tumors of man or tumors remaining after surgical removal of the primary tumor.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Specific and Nonspecific Immunotherapy as an Adjuvant to Chemotherapy

Principal Investigator: Name/Address Performing Organization:	Dr. Frederick Eilber University of California Los Angeles, CA

Contract Number: N01-CB-53941
Starting Date: 6/16/75 Expiration Date: 6/15/79

Goal: To determine if post-surgical adjuvant chemoimmunotherapy is superior to adjuvant chemotherapy in preventing disease recurrence in patients with highly malignant skeletal or soft-tissue sarcomas.

Approach: Patients with osteosarcoma, or grade III soft tissue sarcoma, (rhabdomyosarcoma, synovial cell or anaplastic sarcoma) following complete surgical excision of their tumor are randomized between chemotherpay -- adriamycin, high-dose methotrexate and citrovorum rescue -- and identical chemotherapy plus immunotherapy consisting of BCG and allogeneic tissue culture sarcoma tumor cell vaccine. Chemotherapy is given for one year, the immunotherpay is given for the same year plus an additional year.

Progress: Using conventional therapy all patients entering this study have less than a 20% chance of remaining disease-free for two years. To date, 71 patients are on study. Thirty-six have received chemotherapy alone and 35 have received adjuvant chemoimmunotherapy. Preliminary results with a median follow-up of 17 months indicate that 65% of the patients remain disease-free. Because so few patients have thus far recurred, no statement can be made as to the efficacy of chemotherapy versus chemoimmunotherapy.

Significance to Cancer Research: The significance to this project has been to reduce disease recurrence and to prolong survival in a group of diseases that was previously rapidly fatal. Since the chemotherapy is discontinued after one year, it will be of major importance to determine if those patients who continue to receive immunotherapy will have a more lasting disease-free status than those patients receiving the chemotherapy alone.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $170,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Advanced Cancer of the Breast and Lung

Principal Investigator:	Dr. John Fahey
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-53939
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To evaluate the usefulness of C. parvum and/or BCG as adjuncts to chemotherapy in the treatment of disseminated breast cancer and lung cancer.

Approach: Patients with breast cancer who have received no previous chemotherapy other than hormonal agents will continue to be randomized to treatment with adriamycin and cyclophosphamide with or without weekly subcutaneous C. parvum (protocol BP-2). Patients with breast cancer who have received prior chemotherapy will be randomized to adriamycin and vincristine with or without weekly subcutaneous C. parvum (protocol BP-3). In both instances, patients not receiving C. parvum will be receiving a sterile water placebo. Lung cancer patients will be randomly assigned to chemotherapy (cytoxan, methotrexate, adriamycin and vincristine) with or without BCG (protocol LP-1).

Progress: Ten patients have been entered on protocol BP-2. Protocol BP-3 is just being activated. In the past 12 months, 60 patients entered the lung cancer protocol LP-1 and were randomly assigned to chemotherapy alone, chemotherapy plus BCG (well tolerated), or chemotherapy plus intravenous C. parvum (poorly tolerated). Because of the significant toxicity encountered with i.v. C. parvum, this treatment arm is to be discontinued. Thus far, immunotherapy did not produce a significant difference in response rate, time to progression, or hematopoietic tolerance to chemotherapy in patients who failed to respond to chemotherapy.

Significance to Cancer Research: Cancer of the breast and lung are two of the most common varieties of cancer. Evaluation of immunotherapy as an adjunct to chemotherapy in these tumors has a high priority.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients Without Detectable Disease After Removal of Tumor Containing Lymph Nodes

Principal Investigator: Name/Address Performing Organization:	Dr. Donald L. Morton University of California Los Angeles, CA

Contract Number: N01-CB-64076
Starting Date: 8/8/74 Expiration Date: 10/7/78

Goal: To determine whether immunotherapy with BCG alone or BCG combined with tumor cell vaccine will decrease recurrence rate or prolong survival in melanoma patients with metastases to regional lymph nodes.

Approach: Patients are randomized following surgical lymphadenectomy into 3 groups receiving different postoperative therapy: one group receives no additional therapy, one receives BCG, and one group receives BCG and allogeneic tumor cell vaccine.

Progress: At the present time 134 evaluable patients have been entered into the prospectively randomized clinical trial. There are no significant differences in the recurrence rates among the three arms of the trial at the present time, with the number of recurrences among the three arms being almost identical. However, the proportion of patients who are alive in the groups receiving immunotherapy, 71% for the BCG-alone group and 69% for the BCG-plus-vaccine group, is greater than 58% of patients alive in the control group. Thus, it appears that patients who recurred and were receiving immunotherapy survived longer after recurrence than those in the control group. These differences are highly significanct at the $p < 0.1$ level, after corrections are made for all prognostic factors thus far examined for patients in the BCG immunotherapy arm as compared to controls, but not in the patients receiving BCG and vaccine as compared to controls. Thus, it appears that patients with melanoma metastatic to regional lymph nodes who received BCG during the postoperative period survive longer following recurrence than patients who do not receive postoperative immunotherapy. It is not clear now that immunotherapy lowers the incidence of recurrence in the group as a whole.

Significance to Cancer Research: If the results of this study continue to develop significance as more patients are entered into the study, we can conclude that immunotherapy should become standard therapy for melanoma patients with lymph node metastases.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $362,845

CONTRACT RESEARCH SUMMARY

Title: Active Specific Immunotherapy in Acute Myelogenous Leukemia

Principal Investigator:	Dr. Jacob Zighelboim
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-64006
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the effects of immunizing patients with acute myelogenous leukemia or acute myelomonocytic leukemia with modified allogeneic leukemia blast cells.

Approach: Eligible patients who are in bone marrow remission and receiving consolidation or maintenance chemotherapy will be randomized into three groups according to the dosage and schedule of immunizing cells used. The study will be performed in two sequential phases. Patients randomized during Phase A will receive immunization with lethally irradiated leukemia cells, and those randomized during Phase B will receive vibrio-cholera-neuraminidase-treated leukemia cells.

Progress: Fourteen patients have received injection of allogeneic irradiated cells without untoward complications. Immunological testing revealed a) marked variability in lymphocyte proliferative responsiveness to autologous and allogeneic immunizing myeloblasts; b) circulating remission lymphocytes showed no direct cytotoxicity to immunizing or autologous blast cells; c) remission lymphocytes cocultured _in vitro_ with immunizing blast cells generate mild cytotoxicity to the stimulation blasts but not to autologous blast cells; d) antibodies against immunizing blasts were encountered in 2 patients; e) maintenance chemotherapy was markedly immunosuppressive.

Signficance to Cancer Research: It is important to assess the value of active specific immunotherapy in the treatment of human cancer and to explore in depth the interaction of immunotherapy with immunosuppressive chemotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator:	Dr. William M. Wara
Name/Address	University of California
Performing Organization:	San Francisco, CA

Contract Number: N01-CB-64004
Starting Date: 6/1/76 Expiration Date: 5/31/79

Goal: To document the immunosuppression which occurs following radiation therapy and to determine if a therapeutic advantage can be achieved by the addition of immunotherapy (thymosin) to the conventional treatment given for squamous cell carcinoma of the head and neck region and the esophagus.

Approach: Both groups of head, neck, and esophageal cancer patients will receive radiation therapy and/or surgery as treatment. Patients will then be randomized to receive thymosin or placebo in addition to standard treatment. All patients will be monitored with immunologic tests to measure antibody-mediated and cell-mediated immunity at regular intervals. The data will be analyzed for local disease recurrence, metastatic disease, and survival difference in the patient groups.

Progress: Fifty-five patients with squamous cell carcinoma of the head, neck, and esophagus have been evaluated prior to irradiation and thymosin therapy. Immunity prior to treatment as measured by total lymphocyte count, E and EAC rosettes, lymphocyte stimulation with PHA and in mixed MLC with allogeneic cells, delayed hypersensitivity skin tests, and quantitative serum immunoglobulins was comparable and normal in the 40 control patients, and in the 15 thymosin treated patients. Postirradiation thus was significant depression in cellular immunity in both groups of patients with decreased T and B cell numbers, and depressed PHA and MLC stimulation. At 6 months post-irradiation our preliminary results suggest that the thymosin treated patients may be reversing their immunosuppression by a return of MLC function and positivity of delayed hypersensitivity skin tests.

Significance to Cancer Research: This study will determine the significance of immunosuppression caused by irradiation in head, neck, and esophageal cancer patients and whether the effect of immunotherapy (thymosin) in reversing this immunosuppression has any impact on disease-free interval, survival, and local control of cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $103,450

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Murine Leukemia Using Syngeneic Hybrid Cells

Principal Investigator:	Dr. Edward P. Cohen
Name/Address	University of Chicago
Performing Organization:	Chicago, IL

Contract Number: N01-CB-74195

Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To evaluate the efficacy of syngeneic hybrid cells in the treatment of spontaneously occurring and passively transferred murine leukemia.

Approach: Somatic hybrids of murine leukemia and LM cells, a sustained mouse tissue culture line, are rejected by syngeneic mouse recipients. Mice rejecting such hybrid cells resist in part what are otherwise invariably lethal challenges of the leukemia cells used as one parent in forming the hybrid. Immunity in resistant animals is directed at least in part toward a tumor associated antigen of parental and hybrid cells.

Progress: Cells cytotoxic for ^{51}Cr-labeled leukemic cells are detected in mixtures of spleen cells from syngeneic mice cocultured for 48 hours with hybrid cells. Cells cultured under similar conditions with LM cells or without added cells are unreactive toward leukemic cells.

The cytotoxic effector cells show no reactivity toward ^{51}CR-labeled P815 cells, an unrelated line. The induction _in vitro_ of specific cytotoxicity will provide a more controlled means of investigating the immunity afforded by hybrid cells. The immunogenic potential of hybrid cells in syngeneic mice is under further evaluation. Mice receiving leukemia cells, followed by combination therapy with cytosine arabinoside and methotrexate and then hybrid cells, survive longer than mice receiving either form of treatment alone. Treatment protocols have been established to determine optimum quantities of cytotoxic drugs.

Significance to Cancer Research: The overall objective of this project is to develop a useful therapeutic adjunct to the treatment of human leukemia. Hybrid cells lose their oncogenic potential in immunocompetent hosts; but retain the surface characteristics of neoplastic cells. They become effective immunizing agents.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $77,000

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in Tumor Immunology

Principal Investigator: Drs. Jon R. Schmidtke and Robert Nelson
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-43948
Starting Date: 6/1/74 Expiration Date: 5/31/78

Goal: To study the role of macrophage function in afferent and efferent phases of immune response in both murine and human systems.

Approach: In murine systems: to determine a number of macrophage functions in both normal and tumor-bearing animals as a function of time after introduction of a syngeneic tumor, and to determine if there are changes in macrophage function with progressive tumor growth that may affect the ability of these cells in regulate the immune response to tumors. In human systems: to develop six *in vitro* quantitative assays of macrophage function and to apply them to monocytes taken from both normal and tumor-bearing individuals.

Progress: Assays were developed to determine the ability of macrophages to phagocytose antigen, to measure metabolism of tumor and other antigens, and the ability of macrophages to transfer immunogenicity to protein antigens. Macrophages in tumor-bearing mice do not differ significantly from normal macrophages in their ability to take up, catabolize, and transfer immunogenicity to protein antigens. Macrophages taken from adjuvant (BCG or *C. parvum*) treated donor mice, upon transfer to syngeneic, tumor bearing mice showed significant immunotherapeutic potential in these hosts. Using human cells, six functional tests for monocytes were developed. These include assays for lysosomal enzyme levels, chemiluminescence response, receptor function, migration of monocytes, phagocytic function, and cell surface receptors in both normal and tumor bearing in humans.

Significance to Cancer Research: The apparent discrepancy between the ability of adjuvant treated macrophages to be immunotherapeutic *in vivo* but to inhibit the development of tumor specific cell mediated cytotoxicity *in vitro* is important with respect to adjuvant therapy in humans. This research shows that macrophages activated *in vitro* with adjuvants may be immunotherapeutic.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of *Mycobacterium phlei* in Advanced Lung Cancer, Hypernephroma and Malignant Melanoma

Principal Investigator: Name/Address Performing Organization:	Dr. Gerald J. Vosika University of Minnesota Minneapolis, MN

Contract Number: N01-CB-64002
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: (1) To establish the clinical dose, toxicity, and efficacy of intradermal and intralesional *Mycobacterium phlei* cell wall skeleton (CWS) plus P_3 lipid; (2) to evaluate the immunological effects and mechanism of action of the CWS/P_3.

Approach: Patients with advanced lung cancer, hypernephroma, and malignant melanoma are entered into study. Patients receive either intradermal or intralesional inoculation of the vaccine. The dose is escalated to establish a maximum tolerable level. Tests of lymphocyte and monocyte cell populations and function are being carried out to determine the immunological effects of the vaccine and to correlate this with clinical response.

Progress: During the first year we established that CWS/P_3 could be given in doses up to 1200/600 μg without undue toxicity. Regressions were observed in patients with hypernephroma and malignant melanoma. During the past contract year, the number of patients treated has reached a total of 51. No responses were seen in patients with lung cancer or breast cancer. One patient with hypernephroma of 13 treated has had a good partial response of lung, abdominal and liver metastasis. Fifteen patients with malignant melanoma are evaluable. Six patients had response of injected disease. Four patients had regression of non-injected disease. One patient had regression of visceral (lung) disease. Response to therapy correlated with the initial immune status of the patient. No patient who was immunoincompetant responded.

Significance to Cancer Research: This study demonstrates the efficacy of CWS/P_3 therapy. It further demonstrates for the first time, immunological regression of visceral metastasis in malignant melanoma.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Syngeneic Tumors in Wistar Rats

Principal Investigator: Name/Address Performing Organization:	Dr. Robert W. Baldwin University of Nottingham Nottingham, England

Contract Number: NO1-CB-64042
Starting Date: 6/18/76 Expiration Date: 6/17/79

Goal: To evaluate the relative effectiveness of bacterial immunostimulants including BCG and _C. parvum_ for active specific immunotherapy and adjuvant contact therapy of transplanted rat tumors, especially those reviewed as being relevant models for human disease.

Approach: Two modalities of treatment are being evaluated in susceptible systems, such as carcinogen-induced rat sarcomas, to define optimum conditions. These procedures are then being used to determine their value in the treatment of more clinically relevant systems, e.g., transplants of spontaneous rat sarcomas and mammary carcinomas. In each system the effectiveness of therapy is being assessed against local tumor and metastatic deposits.

Progress: Adjuvant contact therapy in which BCG or _C. parvum_ is administered to contact with tumor cells is an effective modality of treatment of transplanted rat tumors. These approaches have been extended to the treatment of mammary pad transplants of spontaneous mammary carcinomas. Intralesional therapy with BCG or, more effectively, _C. parvum_ (CN 6134) controls growth of the local tumor. Intralesional therapy with _C. parvum_ controls metastatic spread to the regional lymph node and lungs.

Active specific immunotherapy using tumor cell vaccines incorporating BCG or its MER fraction, as well as _C. parvum_, is effective in controlling growth of some tumors. This approach is more limited, however, since only relatively small amounts of tumor can be suppressed. Moreover it depends upon the innate immunogenicity of the tumor so that, while effective with carcinogen-induced tumors, spontaneous sarcomas and mammary carcinomas were not generally responsive to active immunotherapy.

Significance to Cancer Research: _In situ_ stimulation of host responses of both specific and nonspecific types by adjuvant contact therapy has potential for immunotherapy. This suggests that stimulation of nonspecific immunity may be effective for treatment of tumors in other organs and this is being evaluated with both animal and human lung tumors.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $84,500

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunity Induced by Drug (DTIC)-Mediated Tumor Antigens (DMTA)

Principal Investigator:	Dr. Enzo Bonmassar
Name/Address	University of Perugia
Performing Organization:	Perugia, Italy

Contract Number: N01-CB-64054
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: Studies concerning the origin and the properties of new tumor-associated transplantation antigens induced in cancer cells following treatment of tumor-bearing mice with drugs.

Approach: Xenogeneization of tumor cells *in vivo* by treatment of tumor-bearing hosts with dimethyltriazenoimidazole-carboxamide (DTIC). Utilization of the drug-mediated transplantation antigens for antitumor immunotherapy.

Progress: Treatment of lymphoma-bearing mice for a number of transplant generations with DTIC can induce strong DMTA (1976). The results obtained in 1977 show that: a) lymphoma lines antigenically altered by DTIC seem to contain heterogeneous cell population expressing distinct DMTA; b) increased tumor-cell associated immunogenicity can be induced by one cycle of DTIC treatment (drug-mediated increase of tumor immunogenicity, or DMITI); c) primary anti-DMTA cytotoxic responses can be obtained *in vitro*; d) successful immunochemotherapy of parental TATA-positive lymphomas can be attained using sublines bearing DMTA + TATA; e) DMITI produced by DTIC in mice bearing the primary lymphoma cannot elicit transplantation resistance in host mice since DTIC is highly immunodepressive. Moreover no immunological reactivity can be restored by transfer of syngeneic lymphocytes. Restoration of graft responsiveness can be obtained if DTIC treatment is followed by cyclophosphamide (Cy); f) successful immunochemotherapy of mice bearing weakly immunogenic lymphoma can be attained using the following treatment schedule: 1. DTIC (for producing DMITI) - 2. Cy (for producing "vacuum" in host's lymphoid system) - 3. Transfer of syngeneic lymphocytes (which proliferate and restore graft responsiveness against DTIC-induced DMITI) - 4. BCNU chemotherapy (for producing antitumor effects in combination with anti-DMITI immune responses).

Significance to Cancer Research: The possibility of inducing new antigenic targets on cancer cells by treating the tumor-bearing host with drugs could open new perspectives in cancer immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $67,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of C3H Murine Mammary Carcinomas

Principal Investigator: Name/Address Performing Organization:	Dr. Bernard Fisher University of Pittsburgh Pittsburgh, PA

Contract Number: N01-CB-64044
Starting Date: 6/16/76 Expiration Date: 6/15/79

Goal: To improve growth inhibition of murine mammary carcinoma, obtained by combined immunotherapy (*C. parvum*; CP) and chemotherapy (Cyclophosphamide; CY), by adding or substituting immunomodulators and chemotherapeutic agents, and by varying aspects of administration.

Approach: Since intratumor (i.t.) CP has shown superior effectiveness, its optimal dose will be determined. Effort will be aimed at manipulating therapy of a primary tumor to improve distant tumor control. Experiments will evaluate radiation or mitomycin-C plus CP following primary tumor removal. The combination will be used when primary tumors are removed or after i.t. immunotherapy. Studies will evaluate the effect radiation and i.t. immunotherapy on distant tumor. Administration of NSSAs by i.t. and systemic routes simultaneously will be investigated. The effect of altering therapeutic regimens after the response to one regimen has been achieved will be studied.

Progress: Findings using Bru-Pel and Glucan support those obtained with CP, BCG, Tilorone, levamisole and MSF-MB, indicating that an immunostimulating agent alone fails to control tumor growth. The two agents augment CY effectiveness but not to the same degree as CP. Addition of other NSSA to CP and CY failed to improve tumor growth control but increased mortality. I.t. treatment with CP resulted in inhibition or complete regression. Since contact of NSSA and tumor antigen controlled tumor growth better, there may be a biological reason for administering immunotherapy directly into primary tumor *prior* to its removal to control distant metastases. Delay of surgery to permit 4 courses of i.p. CP and CY gave little advantage in tumor control but 4 courses of i.t. CP and CY controlled an untreated tumor and prolonged survival. Systemic CP given following tumor radiation had little effect. Systemic CP and CY following 2000r resulted in tumor growth inhibition, greater than that produced by any of the 3 agents alone or 2 in combination. Radiation of a tumor prior to CY and local CP therapy resulted in greater inhibition of tumor growth and survival. Tumors receiving radiation prior to chemoimmunotherapy responded uniformly to therapy in contrast to the responses observed without radiation.

Significance to Cancer Research: Findings suggest an advantage to delaying primary tumor removal so that it may be used in multimodality therapeutic strategies to control metastatic disease. Administering immunostimulating agents directly into a primary tumor may increase host immune response and eliminate residual tumor, which chemotherapy alone is unable to control.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy of Recurrent Superficial Bladder Cancer

Principal Investigator:	Dr. Donald L. Lamm
Name/Address	University of Texas Health Science Center
Performing Organization:	San Antonio, TX

Contract Number: N01-CB-74192
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled randomized prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: To date 19 patients have been entered into the protocol. They are stratified according to tumor grade and recurrence rate. Although it is too early to make comparisons regarding rate of tumor recurrence, preliminary data suggest that those patients who received BCG have had no serious side effect from this treatment.

Significance to Cancer Research: (NCP Objective 6 Approach 1,4)

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $74,500

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutic Studies with Lymphokine 1788

Principal Investigator: Dr. Ben W. Papermaster
Name/Address University of Texas Medical Branch
Performing Organization: Galveston, TX

Contract Number: NO1-CB-64058
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: Production and characterization of cultured lymphoid cell lymphokine fractions, which induce inflammation and regression of susceptible tumors in animals and man.

Approach: Cell culture supernatants are concentrated and purified by column chromatography and gel electrophoresis and labeled with radioisotopes in order to follow minute quantities. Labelled fractions are then run on 6 M guanidine-HCl columns or in SDS electrophoresis and then tested in vitro and in vivo for antitumor activity.

Progress: New assays for lymphotoxin and MIF have been developed. Purification of ^{3}H-amino acid labelled lymphotoxin was accomplished from human and mouse sources. Quantitative mouse tumor models for evaluating lymphokine immunotherapy have predominantly consisted of the L1210 lymphoma in DBA/2 mice. These have been quantitatively refined by using the Gehan modification of the Kaplan-Meir generalized Wilcoxin technique for calculating survival times. Most recently we have been able to achieve highly significant prolongation of survival time in a chemoimmunotherapy model in which treatment of animals receiving 10^4 L1210 cells IP with methyl-CCNU on the 3rd and 16th day is supplemented with a mouse lymphokine preparation. Addition of lymphokine preparation to the methyl-CCNU schedule on days 3, 7, 10, 13, 16, and 20 prolongs survival over the drug group alone and is significant at $p < 0.001$ and $p < 10^{-5}$ versus controls receiving tumor cells only and lymphokine plus cells only.

Significance to Cancer Research: Lymphokine preparations appear to mobilize inflammatory cells and induce tumor regression. Such preparations may be useful as adjuncts to chemotherapy, and in some cases may induce complete regression by augmenting the natural immune response of lymphocytes and macrophages to the tumor where they have previously become refractory.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Antibody-Drug Conjugates in Immunotherapy

Principal Investigator: Name/Address Performing Organization:	Prof. Ruth Arnon Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-64049
Starting Date: 6/30/76 Fxpiration Date: 6/29/79

Goal: To explore the feasibility of an immunochemotherapeutic approach - the use of antitumor antibodies as specific carriers of anti-cancer drugs for local delivery of the drugs to the tumor site.

Approach: Anti-cancer drugs such as daunomycin and adriamycin are attached covalently either to the IgG fraction of antitumor sera or to purified antitumor antibodies. The cytotoxic effect of the resulting conjugates on the tumor cells is tested both *in vitro* and *in vivo*, and compared to the effect of free drug, free antibodies, their mixtures, or conjugates of the drugs with normal immunoglobulins, and its mechanism of action is investigated.

Progress: The mechanism of action of daunomycin conjugates was explored by the use of radioactive compounds labelled at different positions. Drug conjugates are taken up by the cells in the bound form and thereafter, while inside the cell, the free drug is released to perform its intranuclear activity. The release probably takes place at acidic pH in the lysosomes.

Daunomycin was linked to immunoglobulins by additional covalent methods in an attempt to improve the overall activity of the drug and the antibody in the complex. Of the various conjugates prepared, only that of the N-citraconyl derivative which is sensitive to acidic hydrolysis, preserved the drug activity.

Conjugates of daunomycin and dextran were tested for *in vivo* effects on the Yac lymphoma in A/J mice. The conjugate was shown to compete with the free drug at various stages of the tumor development and with high tumor loads.

Antibodies were prepared in both rabbits and mice against the primary tumor of Lewis lung carcinoma in mice. These antisera reacted with the lung metastatic cells as well as with the primary tumor, as determined by the indirect fluorescence assay. Daunomycin bound to the IgG fraction of these antisera was specifically cytotoxic *in vitro* to both the primary and the metastatic tumor cells.

Significance to Cancer Research: The suggested approach is a combination of a chemotherapy and immunotherapy in an attempt to localize the cytotoxic drug in the tumor site while maintaining low systemic concentration.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Altered Membrane Microviscosity: Effects on Tumor Cell Immunogenicity

Principal Investigator Name/Address Performing Organization	Dr. Meir Shinitzky Weizmann Institute Rehovot, Israel

Contract Number: N01-CB-74193
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To increase the antigenicity of murine tumor cells by altering membrane microviscosity.

Approach: Tumor cells from several mouse tumors will be treated with liposomes of varying cholesterol/phospholipid ratios on fatty acids and their derivatives in attempts to modify membrane composition. Correlation between microviscosity and the degree of exposure of various membrane antigens will be studied. *In vitro* studies will focus on the effect of lipid treatment of tumor cells on the sensitization of spleen cells. *In vivo* work will evaluate the degree of immunity induced by prophylactic injection of altered tumor cells, and ultimately the use of altered tumor cells in therapeutic modes.

Progress: Modulation of the expression of well defined antigens by changing the lipid microviscosity ("passive modulation") has been already observed with the common blood group antigens and the θ antigen of T-cells. Passive modulation of weaker antigens is now being studied. In parallel, *in vivo* and *in vitro* screening of the antigencity of a series of tumor cell lines for further studies has been recently commenced.

Significance to Cancer Research: (NCP Objective 2,6 Approach 1,4) The increase by passive modulation in immunogenicity of syngeneic tumors may be used as a clinical immunotherapy tool.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $63,500

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Tumors Using Immunoresponsive Cells Sensitized In Vitro

Principal Investigator: Dr. Jon C. Palmer
Name/Address Wistar Institute
Performing Organization: Philadelphia, PA

Contract Number: N01-CB-64005
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To use mouse tumor models to evaluate the effectiveness of sensitizing host immunocompetent cells with syngeneic tumor cells by in vitro culture and the use of such sensitized cells in cancer transfer tests of cancer therapy and prophylaxis.

Approach: The highly tumorigenic yet demonstrably immunogenic P815Y tumor of DBA/2 mice and EL4 tumor of C57Bl/6 mice are being used to study the relationship between in vitro and in vivo measures of anti-tumor activities. Spleen cells and thoracic duct lymphocytes from normal and immunized mice are stimulated in vitro with mitomycin C-treated tumor cells. Cytolytic and cytostatic activities of cells recovered from culture are measured in vitro and correlations made with the therapeutic and prophylactic activities of these same populations.

Progress: Culture systems were compared for optimum generation of allogeneic and syngeneic cell-medicated anti-tumor immune responses. The method of culture in tissue culture plates with small volumes of medium containing 2-mercaptoethanol was chosen as simplest and best. Colony formation in soft agar and outgrowth in media following limiting dilutions were developed as measures of anti-tumor cytostasis to complement the short-term radioactive chromium release assay of cytolytic activities. The three in vitro assays of anti-tumor activities were compared and found to agree qualitatively and in specificity for different tumor targets. Several in vivo assays by cell transfer of cell populations sensitized in vivo and in vitro have been performed to measure prophylactic and therapeutic activities. The in vitro and in vivo measures of anti-tumor activities agree for cells sensitized in vitro, but effector cells from in vivo sources can have therapeutic benefit without having demonstrable in vitro anti-tumor activity when measured at the time of transfer.

Significance to Cancer Research: Because of greater possibilities for manipulation and modification and because of separation from host control processes, much greater anti-tumor activities might be induced in host immunocompetent cells by the use of in vitro culture than is possible in vivo. Also, because the immunocompetent cells are temporarily removed from the patient, more vigorous radiotherapy or chemotherapy protocols can be used in cancer treatment than would otherwise be possible.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Intratumoral BCG Immunotherapy Prior to Surgery for Carcinoma of the Lung

Principal Investigator: Name/Address Performing Organization:	Dr. Richard Matthay Yale University School of Medicine New Haven, CT

Contract Number: N01-CB-74191
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To evaluate the therapeutic efficacy of intratumoral BCG prior to surgery for carcinoma of the lung.

Approach: Patients with potentially resectable carcinoma of the lung will be randomly assigned to treatment with intratumoral BCG (administered by transthoracic needle or fiberoptic bronchoscope) two weeks prior to surgery or surgery alone. Survival and disease-free interval will be analyzed in the two treatment groups.

Progress: New Contract

Siginificance to Cancer Research: (NCP Objective 2,6 Approach 1,4)

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 78 Funds: $67,500

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator:	Ms. Diane Hernandez
Name/Address	Cor Bel Laboratories, Inc.
Performing Organization:	Rockville, MD

Contract Number: N01-CB-64024
Starting Date: 11/1/75 Expiration Date: 10/31/78

Goal: Maintain colonies of inbred mice (7,500 animals), inbred rats (800 animals), and rabbits (25 animals) and carry out selected breeding protocols with these animals as specified by the project officer. These animals are to be maintained in support of intramural research programs in the Immunology Branch, NCI.

Approach: Colonies of mice, rats, and rabbits are to be housed and fed according to National Research Council standards. Technical manipulations and breeding are to be carried out as directed by the project officer.

Progress: Performance on this contract has been highly satisfactory. The animal colonies have been established and are being maintained according to National Research Council standards. Animal health has, in general, been excellent, and breeding protocols have been satisfactory. Recordkeeping and transferring of animals to and from the NIH Campus have all been satisfactory.

Significance to Cancer Research: This animal colony is necessary in support of intramural research programs in the Immunology Branch of NCI. Many of these programs are concerned with the immune response to cancer.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $175,200

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. A. H. Johnson
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number: N01-CB-53890
Starting Date: 4/1/77 Expiration Date: 3/31/79

Goal: To study HL-A, B lymphocyte, and other cell surface antigens on cells from patients and normals, to establish correlations between disease categories and these antigens, and to study families wherein more than one member has a particular disease. These studies are designed to explore the genetic contribution of the major histocompatibility complex in man to the disease state.

Approach: Complement-dependent cytotoxicity studies will be performed with cells and sera provided by the project officers.

Progress: HLA-A2 is significantly increased in frequency in about 700 patients with acute lymphocytic leukemia studied at diagnosis. These patients are being followed, and HLA antigen associations with survival will be sought. Over 150 lung cancer patients have been studied prospectively; the data have not been analyzed yet. HLA-A2 is increased in frequency in schizophrenia. There are no significant associations in manic depressive psychosis.

HLA and B-cell alloantigen typing are also being performed on patients with the following disease categories: diabetes mellitus, systemic lupus erythematosus, Sicca syndrome, and acute lymphocytic leukemia patients in long-term remission. In addition, more than 700 typings have been performed on the normal Caucasian and Pima Indian populations to establish the frequency and genetics of serologic reactions to B-cell alloantigens and to establish the number of genes controlling antigens expressed on peripheral blood B cells. In data analyzed to date, selected B-cell antigens appear in greater frequency in Sicca syndrome and systemic lupus erythematosus compared with a normal population.

Sera from melanoma patients injected with BCG plus allogeneic melanoma tissue culture cells have been shown to contain anti-HLA antibodies, indicating that they are indeed being immunized.

Significance to Cancer Research: Establishing correlations of malignancies and other diseases with HLA antigens may identify prognostic factors and lead to etiologic clues. The major histocompatibility complex in man may contain immune response genes. Identification of these antigens and attempts to correlate the presence of particular antigens with identifiable immune response will lead to a better understanding of this phenomenon in man.

Project Officer: Dr. G. Nicholas Rogentine, Jr., and Dr. Dean L. Mann
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $244,943

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address George Washington University
Performing Organization: Washington, DC

Contract Number: N01-CB-64022
Starting Date: 5/1/76 Expiration Date: 7/31/78

Goal: 1) Separation and purification of tumor-associated antigens for studies at NCI of cell-mediated immunologic reactivity to these antigens by skin tests for delayed hypersensitivity and by leukocyte migration inhibition assays. 2) Development of antibodies to isolated melanoma antigens and attempts to establish radioimmunoassays for potential diagnostic use.

Approach: Extraction and purification of antigens from carcinoma of the breast, carcinoma of the lung, malignant melanoma, and Ewing's sarcoma. Separated antigens are provided to Laboratory of Immunodiagnosis, NCI, for in vivo and in vitro tests. Separated antigens from melanoma are also used for development of radioimmunoassays with heterologous antibody.

Progress: During the past year several very large preparations from tissue cultures of breast cancer, lung cancer, melanoma, and Ewing's sarcoma have been fractionated, and the separated antigens have been provided to NCI for skin testing and for testing in leukocyte migration inhibition. Tumor-associated antigens have been detected in the breast cancer, melanoma, and Ewing's sarcoma materials and a number of cell lines for lung cancer have been screened for possible tumor-associated antigens.

A major effort has been made toward the establishment of a sensitive radioimmunoassay for malignant melanoma. Data to date indicate that the assay can detect elevated levels in the sera of many patients with malignant melanoma. However, some elevated values have also been obtained with control sera, and efforts are being directed toward preparing more purified antigens and obtaining antibodies with higher binding affinities.

Significance to Cancer Research: This contract provides an opportunity to study antigens which are recognized by the cell-mediated response of cancer patients. In addition, a radioimmunoassay for melanoma is being developed which potentially may be very useful for immunodiagnosis and monitoring of melanoma.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: 0 Contract will terminate 7/31/78

CONTRACT RESEARCH SUMMARY

Title: Systems Analysis and Informational Service in Registries of Clinical Protocols

Principal Investigator:	Ms. Molly A. Wolfe
Name/Address	Informatics, Inc.
Performing Organization:	Rockville, MD

Contract Number: N01-CB-53888
Starting Date: 5/2/75 Expiration Date: 5/31/79

Goal: To provide analyzed integrated information regarding ongoing clinical immunotherapy protocols to cooperating clinicians.

Approach: Standardized data accumulation and storage methods have been developed for characterizing clinical trials on immunotherapy. Protocols submitted to the Registry are analyzed, indexed, and periodically updated; an annual Compendium of these protocols is prepared and distributed to participating clinical investigators. In addition, an address file of approximately 3,000 physicians is maintained and periodically updated. Periodic reports describing current Registry activities and related topics in immunotherapy are published and distributed to those on the general mailing list.

Progress: The fifth edition of the Compendium on Tumor Immunotherapy Protocols was published in September 1977. Prior to its publication, considerable effort was spent in obtaining accurate update information on the 426 active protocols contained in the Registry, and in the analysis of 153 new protocols. A Cooperative Group Index was established to facilitate the elimination of duplicate protocols from the Registry. In past years, Cooperative Group protocols were frequently submitted independently by several different investigators, and these duplicates were impossible to identify in our manual duplicate-check file. An Evaluation Section was added to the summary format of completed protocols. It includes a brief description of the results of the various protocol treatment regimens of each study. Frequently, this information appears in the Compendium prior to its publication in the medical literature.

Significance to Cancer Research: The International Registry of Tumor Immunotherapy is a valuable source of ongoing clinical trials in immunotherapy. The annual Compendium of Tumor Immunotherapy is a mechanism for a rapid exchange of current information among clinicians engaged in immunotherapy, and further, it may encourage and aid in the design of new protocols.

Project Officer: Mrs. Susan Ficker
Program: Managed by the Tumor Immunology Program in Immunotherapy
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: Approx. $76,000 from ICRDB, OD/NCI

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation, and Preservation of Plasma Cell Tumors in Mice

Principal Investigator:	Dr. Jean Dealy
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Bethesda, MD

Contract Number: N01-CB-92142
Starting Date: 6/23/69 Expiration Date: 5/31/78

Goal: Transplantation, preservation, induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice, and breeding of rare strains of mice, hybrids, and wild mice.

Approach: To maintain a frozen tumor bank of about 1,100 tumor lines of plasmacytomas, B- and T-cell tumors, which is the source of reference tumors for both intramural NCI and outside investigators. To supply tumor lines, tumor products (ascites, serum, urine, purified myeloma proteins), and antisera to NCI for ongoing research. To maintain a special screening bank of 250 typed myeloma proteins derived from plasmacytomas. These proteins are classified for V-isotypes and antigen binding activities. To breed conventional mice for production of various congenic strains and mice used in plasmacytomagenesis experiments, and to maintain these mice during plasmacytomagenesis. To maintain a colony of rare species and subspecies of Mus.

Progress: Contractor has improved its expertise in developing and testing Ig-V-region isotypic and idiotypic antisera for classifying V-regions of the 250 myeloma proteins in the special screening bank. The QUPC52 1 6 DEX idiotype has been characterized and found to be a new immune response marker. Effective facilities for plasmacytoma induction experiments are in full operation. Currently, the role of age on plasmacytomagenesis is in progress. Twenty new congenic BALB/c strains to be used in plasmacytomagenesis experiments are being developed. Each strain carries a different marker (allotype, isozyme Hbg, FV-1, etc.) from DBA/2. Strains are in 5th backcross. Contractor continues production of Mus cervicolor, caroli, shortridgei, pahari and Mus m. castaneus, musculus and molossinus. LBI made 1,450 shipments of mice tumors and products to other investigators. Contract has supported ongoing immunochemical-structural studies in NCI, NIAID, and NIAMD.

Significance to Cancer Research: Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter and David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $141,802

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator:	Dr. Jack Dean
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: N01-CB-63975
Starting Date: 11/1/75 Expiration Date: 8/31/78

Goal: To study the immune responses of cancer patients to tumor-associated antigens and to correlate results of different assays with each other and with clinical status of patients. To provide support for the Laboratory of Immunodiagnosis, NCI, for its detailed studies of cell-mediated immunity to human cancer.

Approach: Immunological tests to evaluate and monitor the immunocompetence of patients and their response to tumor-associated antigens include lymphocyte stimulation by mitogens, recall antigens, and autologous tumor antigens; rosette assays for enumeration of T cells and their subpopulations; leukocyte migration inhibition assays against recall antigens and tumor-associated antigens; and studies of direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity against various human tumor cell lines.

Progress: Serial testing of patients with carcinoma of the lung, breast, malignant melanoma, and Ewing's sarcoma has been continued. In lung cancer patients two immunologic assays have correlated well with clinical status and prognosis. Patients who have developed recurrent disease after surgery have depressed percentages of high affinity rosette-forming T cells and depressed lymphocyte proliferation responses to PHA and to allogeneic leukocytes. Depressed proliferative responses of many patients have been attributable to the presence of suppressor cells, the majority of depressed lung cancer patients having adherent suppressor cells and the majority of depressed breast cancer patients having nonadherent suppressor cells. Tests of autologous proliferative responses of lung and breast cancer patients to tumor extracts have shown that approximately 50% of the patients had reactivity and that this was directed against tumor-associated antigens. Lack of reactivity of some patients has been shown to be due to suppressor cells. In studies of leukocyte migration inhibition to extracts of tumor cells and tissue culture cell lines, indirect assays confirm that the assays were measuring production of the lymphokine, leukocyte inhibitory factor, by specifically immune lymphocytes. In cytotoxicity assays, the major emphasis is on understanding normal cytotoxic reactivity and the possible presence of other types of cytotoxic cells in the blood of cancer patients.

Significance to Cancer Research: These studies are providing important information regarding the usefulness of various assays of cell-mediated immunity for immunodiagnosis and for monitoring the clinical course of cancer patients. In addition, important new information on the mechanisms underlying cellular immunity in cancer is being obtained.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $683,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Biologic Studies of Solubilized Tumor Antigens

Principal Investigator: Dr. Jack Dean
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, MD

Contract Number: N01-CB-63987
Starting Date: 9/29/76 Expiration Date: 9/28/78

Goal: To develop a rapid, reproducible, and specific assay to monitor solubilization and purification of various TSTAs. To study cell-mediated mechanisms using soluble antigens to immunize syngeneic mice.

Approach: The biologic activities of detergent-solubilized and other various chromatographic fractions of plasma membranes bearing TSTA (tumor rejection activity) are studied by lymphocyte stimulation, inhibition of macrophage migration, Winn assay, and delayed hypersensitivity reactivity (in the foot pad). Tumor rejection activity of these materials is also assayed. TSTA activities of chemically induced and of DNA and RNA virus-induced syngeneic neoplasms of the mouse are being studied.

Progress: Detergent-solubilized (NP40 and deoxycholate) and partially purified fractions of TSTA following chromatography on agarose-polyacrylamide columns have shown specific activity in the agarose droplet assay for inhibition of macrophage activity and in the foot pad assay for delayed hypersensitivity reactivity. Nanogram amounts (protein) have shown activity in those fractions known to have tumor rejection activity in the syngeneic mouse. Fractions obtained from Meth-A, a chemically induced sarcoma, and from mKSA, an SV40-induced sarcoma, were specifically active, whereas no specificity could be obtained in these assays using materials prepared in the same way from leukemic cells induced by RNA viruses; these latter neoplasms are continuously releasing infectious virus, and this may be a complication.

In our assays, "immune deviations" such as "overloading," "restricted range of activity" and prevention of cell-mediated immune responses reported by others (Baldwin, Boone, Kahan) are not observed when using the most highly purified soluble or solubilized antigens of Meth-A (chemically induced tumor) or mKSA (SV40-induced tumor).

Significance to Cancer Research: (NCP Objective 3 Approach 5) These studies of the transformation of specific tumor antigens on the cell surface of neoplastic cells will increase our understanding of the mechanisms of cancer induction and maintenance of the neoplastic state and may lead to the prevention and control of neoplasms in man.

Project Officer: Dr. Lloyd W. Law
Program: Tumor Immunology Program
Technical Review Group: Immunology Support (Ad Hoc Review)
FY 78 Funds: $246,268

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator:	Dr. Julie Djeu
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: N01-CB-74093
Starting Date: 2/28/77 Expiration Date: 2/27/79

Goal: Study of natural cell-mediated cytotoxicity of mice and rats and of their immune responses to tumor-associated antigens. Studies on *in vivo* resistance to tumors and correlation with *in vitro* cellular immune responses.

Approach: To study (1) the characteristics of the cellular immune responses of tumor-associated antigens to tumors induced by murine leukemia viruses and murine sarcoma virus, (2) the natural cell-mediated cytotoxicity against tumors of mice and rats, and (3) the factors influencing the levels of this reactivity and the mechanism of cytotoxicity.

Progress: The main emphasis has been on natural cell-mediated cytotoxicity in mice. This reactivity has been found to be augmented substantially by inoculation of various tumor cells, viruses, *C. parvum*, BCG, and other materials. This boosting has been shown to be mediated by induction of interferon. It has been possible to boost reactivity both *in vivo* and *in vitro* by use of interferon, and the effects of interferon or interferon inducers have been blocked by specific antisera. Induction of interferon and consequent augmentation of cytotoxicity of poly I:C has been shown to be dependent on macrophages. These findings have allowed detailed analysis of the role of natural killer cells and macrophages in resistance against tumors. The effects of various chemotherapeutic and immune modulating agents on natural killer activity has been studied as a means of developing regimens to discriminate between natural killer activity and macrophage-mediated anti-tumor activity. Differences in susceptibility between the natural killer cells and their precursors have been found, with the precursors being much more resistant to radiation and several chemotherapeutic agents. In addition, a sensitive assay for antibody-dependent cell-mediated cytotoxicity against mouse tumor cells has been developed, and this has permitted parallel studies of various manipulations on both natural killer activity and antibody-dependent cytotoxicity.

Significance to Cancer Research: Understanding the relationship of *in vitro* assays to host resistance of tumors is an essential element in our ability to use these assays to monitor the clinical response to immunotherapy and to understanding the role of various immune mechanisms in resistance to tumor growth. Recent findings with natural cell-mediated immunity may account for a significant portion of *in vivo* resistance to tumor growth, and may have important implications for immune surveillance.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $279,995

CONTRACT RESEARCH SUMMARY

Title: Melanoma Cell Vaccine in *In Vitro* Assays for Humoral and Cellular Cytotoxicity

Principal Investigator:	Dr. Edwin Matthews
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: N01-CB-53916
Starting Date: 5/9/75 Expiration Date: 5/10/79

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out *in vitro* cytotoxicity tests to measure immune responses of patients to the vaccine.

Approach: (1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy. (2) Carry out tests for humoral and cellular immunity using peripheral blood cells from protocol patients and control normal donors.

Progress: Between January 1977 and December 1977, 8 batches of pooled vaccine were prepared from human melanoma cell lines, UCLA SM-6, UCLA SM-12 and UCLA SM-14. All of these vaccine preparations met quality control tests and have been used in the melanoma immunotherapy protocol. During the same period, approximately 4,800 assays for cellular immunity were carried out using 800 blood samples from patients receiving the vaccine, patients in control groups of the protocol, and normal donors. In addition, 450 serum samples were frozen and stored in approximately 5,000 vials. Results of *in vitro* testing have demonstrated that (1) greater than 90% of melanoma patients receiving the melanoma vaccine generated cellular and humoral responses against one or more of the vaccine cell lines; (2) melanoma patients, except for those receiving chemotherapy, have a normal capacity for generating cytotoxic cellular responses *in vitro*, and to this extent are not immunosuppressed; and (3) treatment of melanoma patients with a nonspecific immunostimulant (BCG alone or BCG and vaccine) has not increased the average natural killer or K-cell activities in these patients.

Significance to Cancer Research: (NCP Objective 6 Approach 1) The melanoma cell line vaccine will be used in a clinical immunotherapy trial.

Project Officer: Dr. William D. Terry and Dr. John R. Wunderlich
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $226,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Rhesus Monkey Histocompatibility Studies

Principal Investigator:	Dr. Bruce Maurer
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: N01-CB-64016
Starting Date: 11/1/75 Expiration Date: 6/30/78

Goal: To establish and maintain a colony of pedigreed rhesus monkey families for the study of the genetics of transplantation and as an eventual source of donor-recipient pairs for transplant experiments.

Approach: Sera raised by allo-immunization of selected monkeys are analyzed for serologically defined antigens by microcytotoxicity typing of families and large random unrelated populations. Monkeys are typed for MLC locus antigens by performing mixed lymphocyte cultures using responder lymphocytes primed in MLC to the MLC locus antigen of a sibling differing by one RhLA haplotype. The method of Mann _et al_. is used to to identify B-lymphocyte alloantigens serologically. Breeding is designed to enlarge existing families of full siblings. The method of Gillis and Smith is used to establish lines of lymphocytes cytotoxic for RhLA alloantigen-bearing cells.

Progress: Analysis of about 300 alloantisera has resulted in improved definition or new identification of a total of 24 RhLA alloantigens. During the current contract year, analysis of several new sera by methods of population genetics and by absorption led to the identification of four of these 24. Optimization of a system for primed lymphocyte typing (PLT) has been completed. Four different primed lymphocytes have been shown to detect RhLA-linked determinants. Comparison of primed lymphocytes with homozygous typing cells indicates the two methods may not lead to identification of the identical determinant. Typing of a random population of monkeys with a panel of 57 sera detecting antigens exclusively present on B-lymphocytes has been completed. Seven antigens have been identified, and family studies indicate at least four are linked to RhLA. Specifically cytotoxic cells have been maintained in culture for greater than 40 days. Various methods of conditioning medium for support of these cultures and screening of responder-stimulator pairs for long-term proliferative potential are being evaluated.

Significance to Cancer Research (NCP Objective _6.1A_ Approach _6.6_) Bone marrow transplantation may allow more intensive cancer chemo-, radio- or immunotherapy than is presently possible. Transplantation of other organs may be useful for replacement of tumor-ravaged organs.

Project Officer: Dr. J. R. Neefe, Jr.
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $161,215

CONTRACT RESEARCH SUMMARY

Title: Tumor Virus Expression in Normal and Transformed Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth Blackman Meloy Laboratories Rockville, MD

Contract Number: N01-CB-74139
Starting Date: 6/15/77 Expiration Date: 6/14/79

Goal: To study expression of tumor viruses in tissue culture and in animals.

Approach: Viral biochemical, serologic, and biological parameters are monitored in different systems to define the regulatory mechanisms of tumor virus expression and to relate the expression to tumor production.

Progress: A quantitative assay for transfection of mammalian leukemia and sarcoma viruses has been developed for use in studies of control of viral expression.

Infectious unintegrated Harvey sarcoma virus DNA has been found to be a double stranded molecule of about 4×10^6 daltons. Sarcoma viral DNA can transform in the absence of helper viral genomes. Also, bulk cellular DNA from normal AKR mouse cells, which is not infectious under standard transfection conditions, is infectious if recipient cells are treated with 5-iododeoxyuridine. This procedure may be useful in establishing the infectious potential of other endogenous viral genomes.

In addition, glucocorticoids have been found to induce foci of morphologically transformed cells in a morphological untransformed mink cell line which contains the Moloney murine sarcoma viral genome. The continued presence of glucocorticoid is required for the maintenance of foci. The induction of foci correlates with intracellular increases in sarcoma virus-specific viral RNA and in murine viral p30 protein.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Douglas R. Lowy
Program: Immunology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $85,350

CONTRACT RESEARCH SUMMARY

Title: National Cancer Institute Immunodiagnostic Reference Center

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth Kortright Meloy Laboratories, Inc. Springfield, VA

Contract Number: N01-CB-63976
Starting Date: 7/11/75 Expiration Date: 7/10/78

Goal: To develop new assays for tumor-associated antigens, to use immunochemical techniques for the diagnosis of cancer, to evaluate humoral immune responsiveness of patients with cancer.

Approach: To use sensitive radioimmunoassays to quantitate human, monkey, and mouse alpha-fetoprotein and human chorionic gonadotropin in serum, to evaluate these assays both as diagnostic techniques for testicular, ovarian, and hepatocellular cancer, and as a means of monitoring the clinical response to therapy. Human lung tumors are being fractionated to isolate lung tumor specific antigen reactive with cancer and to establish a radioimmunoassay for this antigen. The contractor performed tests of humoral competence of patients with cancer or immunodeficiency diseases.

Progress: Alpha-fetoprotein and HCG assays have been shown to be of great value in staging germ cell tumors of the testis and as a tool to monitor the chemotherapy of testicular germ cell and hepatocellular tumors. These assays are being performed in collaboration with eight groups in monitoring the treatment of patients with hepatocellular or testicular germ cell tumors other than seminoma. In all cases in which the marker was elevated, but where there was no clinical evidence of disease, a positive marker always reflected occult residual tumor. A human lung tumor extract has been isolated and reacts with antiserum against human lung tumors but not against normal lung tissue. A radioimmunoassay has been established that can detect very small quantities of this antigen. This assay has been utilized in efforts directed toward establishing an immunodiagnostic test for human lung cancer. Humoral antibody assays have been established for ten antigens and these antibody assays are of critical importance in defining immune defects in patients with high incidence of neoplasia.

Significance to Cancer Research: (NCP Objective 5 Approach 4) These studies are helping to establish the usefulness of these multiple immunodiagnostic tests in the diagnosis of cancer, or as a means of monitoring patients during and after therapy.

Project Officer: Dr. Thomas A. Waldmann, Dr. R. Michael Blaese, Dr. Robert McIntire
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: $310,000 (estimate)

CONTRACT RESEARCH SUMMARY

Title: Preparation of Reagent Antisera and Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Howard M. Grey National Jewish Hospital and Research Center Denver, CO
Contract Number: N01-CB-53912	
Starting Date: 5/15/75	Expiration Date: 5/15/78

Goal: Preparation of monospecific fluorescent-isomer conjugated IgG $(Fab')_2$ antibodies to all heavy and light chain classes of human and mouse immunoglobulins and human T-lymphocytes.

Approach: To purify all classes of human and mouse heavy and light chains by immunochemical techniques. To prepare solid-phase absorbed antisera to each of these antigens. The IgG fractions of such antisera will be isolated, enzyme digested to make $(Fab')_2$ fragments, and conjugated to flourescent isomers. A variety of cell types will be used for both immunization and absorption to make human T-cell-specific antisera. The antibodies will then be further processed as for the anti-Ig reagents.

Progress: Whole rabbit antisera (approximately 100 ml) with specificity for each of the human immunoglobulins (including antisera with λ and κ specificity) have been prepared. Similarly, antisera with specificity for each of the mouse immunoglobulins, including IgG, IgG_1 IgG_2 and IgG_3 as well as mouse λ chain, and antisera with specificity for mouse IgG_2 prepared against myeloma PC5 having κ specificity have been prepared.

A variety of $(Fab')_2$ fluorescein-labeled rabbit antisera with specificity for human and mouse immunoglobulins were prepared. This completes the workscope of the contract with the following exceptions: a) additional amounts of anti-human IgM, IgA and IgD are required to bring the amount of each of these antisera supplied to approximately 75 mg; b) small amounts of each of the purified human immunoglobulins are to be supplied; c) T-cell-specific antisera are to be supplied. To date, rabbits have been immunized with monkey thymus cells. The resultant antiserum has been subjected to DEAE cellulose chromatography and the IgG obtained has been fluoresceinated. The purified, labeled antiserum thus obtained was then absorbed with RBC stroma, CLL cells and lymphoblastoid cell lines to obtain an antiserum that reacts only with a portion of human peripheral blood cells. Further work must be done to assure that the antiserum obtained is in fact T-cell specific.

Significance to Cancer Research: This contract will provide specialized antisera which are required for cell surface studies important in the Tumor Immunology Program.

Project Officers: Dr. Howard B. Dickler and Dr. Warren Strober
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 78 Funds: 0

ANNUAL REPORT
Breast Cancer Program Coordinating Branch
October 1, 1977 - September 30, 1978

The Breast Cancer Task Force Committee continued to implement and extend the research contract program for obtaining baseline studies in experimental biology, in epidemiology and in clinical studies related to diagnosis and treatment of the disease for nine months of the year. For the remaining period the major functions of the Committee have been to review the total breast cancer program supported by grants and by contracts, and to provide advice both on new project areas for development and methods for stimulating investigator-initiated grants. Staff support for the remaining contract projects and for the newly acquired grants program has been provided by the Breast Cancer Program Coordinating Branch. A new section on information has been added to the Branch in order to make available to the Committee members, the staff, and the investigators in the field summaries of the on-going studies in breast cancer on a monthly basis.

In late 1977 fifteen new projects were advertised by the Task Force program, over 90 proposals were received in response to 14 of these in FY-1978. In addition ten unsolicited proposals and two letters of intent were received. All proposals were evaluated for their science by one of four groups of Committee members selected for their expertise related to biology, epidemiology, diagnosis and treatment of the disease. In FY-1979 there will be approximately 90 contracts and six Cancer Research Emphasis Grants (CREG's) funded. No new projects have been formulated for advertising therefore the contract program will be phased down in the next fiscal year through termination of on-going ones and none to be initiated. The CREG's are in their terminal year. Over 550 grants related to breast cancer are presently funded by the Cancer Institute.

Although the Breast Cancer Task Force has been converted to an advisory body their goals will remain essentially those of the past, namely, to promote studies of the etiology of the disease, identification of high risk populations, new diagnostic methods, prevention of occurrence and recurrence of the disease, and seeking new treatment modalities. Contracts related to the goals are listed below by targeted approaches, each contract is referenced by number and those terminating during FY-1978 are so designated with a T following the number. A summary of each contract follows this report.

I. Studies of surface glycoproteins of normal and neoplastic mammary cells, basic studies related to metastasis: 63984, 64069, 4 CREG's - Roswell Park, Tufts University, Oklahoma State University and University of Alabama.

II. Interaction of neoplastic mammary cells with other cell populations: 23863T, 43907T, 63983, 63986, 74089.

III. Response to and dependence on hormones of mammary tissue in the various stages of the development of the gland: 33883, 33907T, 53905T, 63985, 74105, 74091, 74189.

IV. Predicting biological behavior of breast cancer and preneoplastic lesions, human and animal investigations: 23862, 43900, 43969 74099, 74100, 74107, 74118, 84223, 84226.

V. Association of breast cancer with specific risk factors: 53968, 53884, 63997, 74097, 74098, 74104, 74110, 84230, 84231, 84238, 84274.

VI. Studies of high risk factors: 43940, 44003T, 53883, 63996, 63998, 74101, 74102, 74202.

VII. Immunological characterization of normal and neoplastic mammary cell populations: 33905T, 33908, 43904T, 53904, 74087, 74092, 84224, 84228.

VIII. Search for markers for breast cancer: 23871T, 53853, 53854T, 53855, 74086, 74206, 84222.

IX. Pathophysiology of solid mammary carcinoma: 33856, 53903, 63980, 63981, 74108. 74109, 84227, 84239.

X. Diagnostic methodology: 43905, 43869, 43993, 44011T, 44012, 64041, 84273, 2 CREG's - University of Texas (M.D. Anderson) and Thomas Jefferson University.

XI. Predicting response to chemotherapy: 43899, 74083, 74203, 74204, 74205.

XII. Surgical adjuvant therapy and therapy of Stage IV cancer: 33899, 43917, 43990, 53851, 53917, 64000, 84221.

XIII. Prevention of neoplastic transformation: 43906T, 74090, 74207.

XIV. Growth and preservation of normal and malignant cells: 23875T, 33898T, 74094, 74188.

XV. Service and support for contract investigators and grantees: a. estrogen receptor (ER) assays - 43867; b. data collection and analysis of ER and marker studies - 74140; c. human cell culture bank and animal tumor bank - 74175; d. preparation of antibodies to collagen and procollagen - 84225; e. storage of human sera: 23879, 43278T, 74137, 74213, 74138; f. purification of estrogen receptors in quantity - 64074.

The Task Force Committee met on three different occasions during the year to hear project presentations by principal investigators representing the programs of experimental biology, epidemiology, diagnosis and treatment. Thirty-four contract supported investigators and six investigators supported by CREG's presented their work to members of the Committee, visitors, and NIH - NCI staff in these open sessions. A discussion period followed each persentation. Following each day of presentations the four working groups met for evaluation of contract renewal proposals, responses to the RFP's, and establishing priorities for funding the new projects.

A major undertaking within the Branch resulted in collecting all publications from scientific journals, monographs, books, etc., in which contract projects have been reported since 1972. Over 200 articles have been bound in four volumes and indexed, these represent the accomplishments of the Task Force during this period. The new Information Section of the Branch has initiated the monthly comprehensive compilation of titles and summaries of the latest papers published in the field of breast cancer. The papers are categorized by project objective, for example, etiology and epidemiology, diagnosis and detection, general biology, pathology, therapy and mortality, etc. These compilations will be made available to active investigators in the field of breast cancer in the coming year.

PUBLICATIONS

Gullino, P. M., Taylor, D. J. and Levine, B. S.: Breast Cancer Task Force Program and Related Projects. Bethesda, MD. January 1978.

CONTRACT RESEARCH SUMMARY

Title: Immunological Studies of Human Mammary Carcinoma

Principal Investigator: Dr. Donald M. Marcus
Name/Address Albert Einstein College of Medicine
Performing Organization: Bronx, NY

Contract Number: N01-CB-23871
Starting Date: 6/20/72 Expiration Date: 6/19/78

Goal: (1) to identify antigens associated with breast cancer and to develop a quantitative radioimmunoassay (RIA) for use in immunodiagnosis of breast cancer; (2) to study the correlation between in vitro assays for cell mediated immunity to breast tumors and the clinical status and course of patients, and to identify antigens that elicit cellular immunity.

Approach: (1) to develop antisera specific for tumor-associated antigens, purify the antigens by immunoadsorbent and biochemical RIA; (2) to make extracts of primary breast tumors obtained at the time of surgical resection and to test autologous and allogeneic extracts against the lymphocytes of the patient by three assays: (a) a viral plaque assay developed by Dr. B. Bloom; (b) inhibition of a leucocyte migration (LMI): and (c) incorporation of ^{3}H-thymidine.

Progress: The standard RIAs used to determine serum ferritin do not adequately measure acidic isoferritins. We devised a solid phase RIA that was relatively specific for acidic ferritin and assayed breast cancer sera examined previously with the standard RIA. The assay for acidic isoferritins was negative in most patients with breast cancer, and is not useful clinically for diagnosis or evaluation.

We found previously that 3 M KCl extracts of breast tumors and fractions obtained by gel filtration gave positive LMI tests in about 50% of patients with breast cancer. In order to obtain a larger and more reproducible source of antigen, we tested extracts from the breast cancer line MCF-7. Our preliminary experiments revealed that unfractionated extracts and a fraction obtained by gel filtration gave positive tests, and we are now preparing larger quantities of MCF-7 cells for more extensive purification.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: K. Robert McIntire, M.D., Bernice Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 2/5/76

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Harold E. Bowman
Name/Address Butterworth Hospital
Performing Organization: Grand Rapids, MI

Contract Number: N01-CB-74213
Starting Date: 9/15/77 Expiration Date: 9/14/79

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients, benign disease patients, and controls, to be used in a search for and verification of new breast cancer markers.

Approach: 30 cc of blood will be collected from breast cancer patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions prior to surgery. Another specimen will be collected, when feasible, 5-10 days post-mastectomy from the same patient. Serum specimens will be stored at -70°C then shipped to NCI designated blood bank facility with approprate clinical data.

Progress: The program has been presented to the surgery sections in each participating hospital. Surgeons who perform 95% of all breast biopsies in the participating hospitals have signed letters of agreement allowing their patients to enter directly into the study. In the first four months of the official operation 146 patients have entered the study; 29 of these patients had malignant disease; 1701 vitals containing serum specimens have been shipped to the central storage facilities at Mayo Clinic; of these vials only 10 or 0.5% have been unsatisfactory for research purposes.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ihor Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $21,450 Site Visit Date 5/17/78

CONTRACT RESEARCH SUMMARY

Title: Biologic Markers in Breast Cancer: Patient Resource

Principal Investigator:	Dr. Ned D. Rodes
Name/Address	Cancer Research Center
Performing Organization:	Colombia, MD

Contract Number: N01-CB-74138
Starting Date: 9/1/77 Expiration Date: 8/31/79

Goal: To serve as a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls, to be used in a search for, and verification of, new breast cancer markers.

Approach: 30 cc of blood will be collected from all Breast Cancer Detection Demonstration Projects screenees (approximately 600 per month). One 30 cc postoperative specimen will also be collected from post-mastectomy cases within the first month. Another specimen will be collected from previous donors at one year anniversary. All blood will be stored at -70°C, then shipped to an NCI designated blood bank facility with appropriate clinical data. This project will consist of a two-year collection and a three-year follow-up period.

Progress: In 68 days of collection (December 9, 1977 - April 3, 1978) from participants in the BCDDP, this unit has interviewed 1,006 subjects and fully processed 958 subjects to provide 8,315 samples for research purposes. This represents approximately 47.2% of BCDDP subjects. Of these samples, there is an average acceptability by the Mayo Blood Bank of 99%. With processing time for the clinical form and with the present serum staff, a conservative number of subjects anticipated per day for this project would be 20. From this, it is exptrapolated that approximately 5,200 subjects will be screened over the next year and that approximately 54,600 specimens of 1.1 ml each will be provided for the Breast Cancer Task Force markers research program.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ihor Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds; $63,350 Site Visit Date: 5/18/78

CONTRACT RESEARCH SUMMARY

Title: Biologic Characterization of "Premalignant" Human Mammary Epithelial Hyperplasias

Principal Investigator: Dr. Kenneth Scott McCarty, Jr.
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number:
Starting Date: Expiration Date:

Goal: To characterize human mammary "premalignant" hyperplasias biologically, biochemically, and immunologically in a search for markers of neoplastic transformation.

Approach: Specimen material will be derived from 200 subcutaneous mastectomies (50 normals included here), 150 biopsies with benign lesions and non-carcinomatous areas from 150 mastectomies performed for breast cancers over a period of three years. Lesions will be classified morphologically by light and electron microscopy. Estrogen and progesterone receptor analyses will be done. Three approaches to the evaluation of immunological markers are proposed: (1) immunoglobulin localization of IgA, IgM, and IgG; (2) presence or absence of K casein, α lactalbumin and carcinoembryonic antigen (CEA); and (3) assessment of cell surface changes by the absence or presence of ABO blood group antigens and/or the unmasking of component antigens as assessed by fluorescent antibody immunohistology. In addition, endogenous peroxidase and thymidine labeling indexes (TLI) will be evaluated. Tissue culture of selected hyperplastic lesions will be carried out to compare the effects on TLI of hormones added to the media (E_2, Prg, Prl, DHT).

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $69,524 Site Visit Date: 5/9/78

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator; Name/Address Performing Organization:	Dr. Fearghus O'Foghludha Duke University Durham, NC

Contract Number: N01-CB-44011
Starting Date: 6/28/74 Expiration Date: 6/26/78

Goal: To evaluate the relative merits of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: Duke University's portion of this inter-institutional study will be subdivided into physics and clinical areas. Duke's physics staff will provide spectrometric services so that xerographic and film modalities can be compared under known spectral conditions. The effects of the radiation spectrum as modified by kV, filtration, and waveform, on image quality and on dose, will also be studied, and the results will be used to determine optimum techniques. In the clinical area, staff will participate in subjective inter-institutional evaluation. In addition, cephalocaudal images made at Duke will be furnished to the Diagnostic Radiology Committee of the National Cancer Institute for review by an advisory board.

Progress: Physics: Elaborate spectroscopic facilities were set up to investigate exposure, mAs and HVT as a function of anode material, filter and waveform, and to study spectral simulation. Certain auxiliary experiments (e.g. efficiency determinations) have led to the development of techniques and of apparatus that have been used in other studies such as trans-molybdenum anode work, internal and external breast dosimetry, and measurement of basic data such as receptor sensitivity. In conjunction with Dr. Malsky of Guttman Institute, studies of dosimetry were also carried out using multipoint TLD measurements. A rapid scanning reader for large area TLD dosimeters has been developed.

Clinical: The clinical aspects of this study were transferred to Hermann Hospital and still await completion.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/31/77

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography--A Comparative Study

Principal Investigator: Dr. Philip Strax
Name/Address: Guttman Institute
Performing Organization: New York, NY

Contract Number N01-CB-44012
Starting Date: 6/28/74 Expiration Date: 6/27/79

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences that may suggest the use of one method over the other in certain situations.

Approach: Characteristics of each process are being assessed by way of radiographic contrast, latitude, sensitivity, and modulation transfer function.

Progress: Clinical: A total of 1,330 women have entered the study since its inception. Since November 1, 1976, Kodak AA type film has been eliminated because of the comparatively high radiation dose required, and all subsequent patient entries received only xeromammograms plus either Low Dose #2 or Min-R film-screen combinations. All films are in process of evaluation per the image evaluation form created for this purpose. Patient accrual has terminated as of June 1, 1977. Biopsy data is now being obtained for operated cases. All patients will be contacted to ascertain subsequent development of breast cancer. Physics: The physics efforts at the Guttman Institute was to ascertain the radiation levels with AA, Low Dose, and Xeromammography receptors employing the conventional filtrations as normally found for the three institutions participating in this study. (Guttman, Duke, and Anderson) using both molybdenum and tungsten targets. Air, phantom, and skin entrance dose levels were to be compared as well as the kVp, mA, mAs. at each of the institutions. For the air output measurements at various target-to-skin distances a Victoreen 415 A and B intercomparison air ionization chambers were employed with a Keithley electrometer and digital readout. For the kVp, mA and mAs readings a Machlett bleeder circuit was used coupled to a storage oscilloscope and the observed bleeder circuit was used coupled to a storage oscilloscope and the observed nominal vs. the bleeder circuit values were recorded. Phantoms: for skin entrance values for varying breast sizes and the institutions' physical parameters (kVp, mAs, target-to-skin distances) with and without compression a 3M phantom was employed. Later studies also employed Mix-D phantom material supplied by the Duke University Physics Group. For skin entrance measurements, thermoluminescent dosimetry chips were used. These physics studies have now terminated, and data are being analyzed.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Diagnositc Radiology Committee
FY 78 Funds: 0 Site Visit Date: 3/31/77

CONTRACT RESEARCH SUMMARY

Title: Isoproteins in Normal, Benign and Malignant Breast Tissues

Principal Investigator:	Dr. Doris Balinsky
Name/Address	Iowa State University
Performing Organization:	Ames, IA

Contract Number:
Starting Date: 8/1/78 Expiration Date: 7/31/79

Goal: To detect, quantitate, and characterize various isozymes or isoproteins in normal, benign and neoplastic breast tissue in an attempt to identify "tumor markers" which may have significance in the early detection of breast cancer or identification of patients with occult dissemination after local treatment.

Approach: A number of enzymes including lactate dehydrogenase, malate dehydrogenase (G6PD), hexokinase, pyruvate kinase, phosphofructokinase, aldolase, alkaline and acid phosphatases, esterases, etc. will be assayed in human normal, benign, malignant and metastatic breast tissues (50 each annually). Patterns of several of these enzymes of interest will be examined in these same tissues. Subsequently these patterns will be examined as well in the sera of normal and breast cancer patients. If the altered isoproteins are isoenzymes, they will be purified from both normal and neoplastic breast tissues and characterized. These studies could possibly give a lead to the altered metabolic status of the tumor. If the isoprotein is non-enzymic, it will be purified and antibody made to it for use in quantitative radioimmunoassay. These data will be correlated with data on estrogen and progesterone receptors on the same material and with histopathologic and clinical data.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $79,800 Site Visit Date: 2/28/78

CONTRACT RESEARCH SUMMARY

Title: The Use of Ultrasound in the Diagnosis of Breast Cancer

Principal Investigator:	Dr. Barry Goldberg
Name/Address	Jefferson Medical College
Performing Organization:	Philadelphia, PA

Contract Number: N01-CB-84273
Starting Date: 5/1/78 Expiration Date: 4/30/79

Goal: To develop characteristic ultrasound patterns of benign and malignant breast pathology in order to evaluate the efficacy of high resolution ultrasonic equipment in the detection of early breast cancers.

Approach: Two different ultrasonic machines, the Octoson B-scan and the Johnson and Johnson real-time scanner, will be clinically evaluated. About 100-150 patients coming to surgery for suspected breast carcinoma will undergo a preoperative X-ray mammogram, physical examination and ultrasonic examination. There will be repeat examinations of the excised breasts. In addition, 8-10 biopsy cases/week recommended for biopsy or aspiration of suspected cysts will be similarly examined. Five hundred asymptomatic patients will also be enrolled per year. Ultrasonic patterns will be correlated with histopathology and X-ray mammograms in both in vitro and intact breast tissues. A secondary objective will be to advance the state of art of detection of breast cancer by incorporating ultrasonic wave form signal analysis and acoustic signaling techniques in a computer learning procedure under subcontract.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., Thomas Shawker, M.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $200,000 Site Visit Date: 12/12/77

CONTRACT RESEARCH SUMMARY

Title: Immunologic Cross-Reactions Between Breast Cancer and Mammary Tumor Virus

Principal Investigator:	Dr. Grace B. Cannon
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: N01-CB-74087
Starting Date: 7/1/77 Expiration Date: 6/30/79

Goal: To perform the leukocyte migration inhibition (LMI) assay for the detection of cell-mediated immunity (CMI) against mouse mammary tumor virus (MMTV) and associated glycoprotein (gp-52) antigens in order to ascertain the usefulness of the LMI assay for the early diagnosis, monitoring, and prognostic predictability of patients with breast cancer.

Approach: Peripheral blood leukocytes from breast cancer patients (prior to biopsy and post masectomy), benign disease patients (pre and post biopsy), patients with other types of cancers, patients with gynecological disease, and healthy female controls will be tested under code in the LMI and MMTV, gp-52, and a 3M KCl extract of the breast cancer cell line MCF-7.

Progress: A higher proportion of presurgical breast cancer patients (7/10, 70%) were reactive to the viral antigens gp-52 and MMTV when tested with these antigens simultaneously as compared to the presurgical patients with fibrocystic disease (2/23, 9%) and fibroadenoma (0/10). This reactivity was based on the level of reactivity of normal females (1/12, 8%) and presurgical gynecological patients (1/15, 7%) to these antigens. MCF-7 was discriminatory between breast disease patients and normal individuals, but not between benign breast disease and breast cancer patients. Thus, reactivity to the viral antigens may be a potential tool for the differential diagnosis of breast cancer, while the reactivity to MCF-7 may assist with diagnosis of breast disease. A low proportion (4/22, 18%) of breast cancer patients were reactive to the viral antigens when tested simultaneously, which raises the possibility that serial testing with these antigens may be useful for determining prognosis.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $132,225 Site Visit Date; 1/11/77

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Computerized Tomographic Mammography

Principal Investigator: Name/Address Performing Organization:	Dr. John J. Gisvold Mayo Foundation Rochester, MI

Contract Number: N01-CB-64047

Starting Date: 6/30/76 Expiration Date: 12/29/77

Goal: To evaluate clinically the ability of computerized tomographic mammography (CTM) to detect early breast abnormalities and compare its effectiveness with physical examination and mammography in individual differential diagnosis.

Approach: A prototype computerized tomographic machine specifically designed to examine the breast has been built by GE and is now functioning at Mayo. As many patients as possible, who are scheduled for breast biopsy and who have had mammography and CT mammography, will comprise the study population. This study population will determine if CT mammography is a feasible diagnostic examination technique for detection of early breast cancer, since the technique is at a lower radiation dosage than is used for current mammographic techniques. For the remainder of the study, patients will be examined both before and after the intravenous injection of iodinated contrast material.

Progress: Of 452 patients examined thus far, 304 have undergone biopsy. Of these, 172 proved benign and 127 were malignant. For the malignant lesions, 60 were done without contrast material injection. Mammography correctly identified 90% of these, while CTM identified 68.3%. Another 62 patients with malignancies were injected with 50 cc of contrast material; mammography correctly identified 87% of these, while CTM correctly identified 84%. We are now doing examinations by injecting 300 cc of contrast material by infusion. Since doing this, 10 lesions were biopsied (5 benign and 5 malignant). Neither mammography nor CTM has missed any of these lesions. The following conclusions have been drawn: (1) CTM cannot be considered a screening device of the need to use contrast material to obtain results equaling mammography; (2) CTM may be a meaningful diagnostic tool in evaluation of problematic breasts, especially breasts dense with benign dysplastic disease; (3) CTM can be done using a low radiation dose, but the dose is doubled when scans are needed both before and after the injection of contrast material; and (4) the future potential of CTM is still uncertain; greater experience is needed using an infusion of 300 cc of 30 percent of contrast material (75% HYPAQUE).

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.

Program: Breast Cancer Diagnosis

Technical Review Group: Breast Cancer Task Force Committee

FY 78 Funds: 0 Site Visit Date: 2/10/77

CONTRACT RESEARCH SUMMARY

Title: Breast Diagnosis: Quantitative Imaging by Ultrasound

Principal Investigator: Name/Address Performing Organization:	Dr. James F. Greenleaf Mayo Foundation Rochester, MN

Contract Number: N01-CB-64041
Starting Date: 6/30/76 Expiration Date: 12/31/78

Goal: To determine if images representing quantitative distributions of basic mechanical tissue properties or acoustic parameters of tissue such as velocity, attenuation, reflection, impedance, and scattering can be useful in breast cancer diagnosis and of more value than images representing tissue interfaces and geometries such as B-scans.

Approach: A transverse radial scanning system has been designed and constructed which acquires ultrasound data from excised tissues and patients in several modes including echos for compound B-scans, time-of-flight, and attenuation data for mathematical reconstruction. The ability of acoustic tissue characterization to detect and distinguish excised normal and diseased tissues is being studied by comparing results from visual evaluation and computer-aided feature extraction and pattern recognition of the various acoustic images with computer encoded histologic characteristics obtained from microscopic evaluation of tissue sections removed from the intact organ. Initial studies have begun of normal and diseased breasts in vivo in selected patients and the resulting acoustic images are being compared to histology obtained from breasts of those women who undergo mastectomy.

Progress: Signal preprocessing systems and scanner control electronics have been designed and constructed. Software programs for data acquisition, analysis, and display have been written and documented. Tomograms of acoustic speed of sound have been obtained from coronal planes through breasts obtained postmortem and in vivo. Two-dimensional distributions of acoustic speed are calculated using computer-assisted tomography. One hundred eighty-one regions from 92 acoustic speed tomograms obtained in vivo have been compared to histology of the breasts after mastectomy. Pattern recognition methods were applied to the data resulting in a cancer specificity of 86% and a sensitivity of 92% in the training set. Results should be improved with addition and attenuation measurements. Pattern recognition methods are being applied to a test set of acoustic tomograms which are being obtained from patients scheduled for biopsy.

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $40,000 Site Visit Date: 2/11/77

CONTRACT RESEARCH SUMMARY

Title: NCI Sera Bank Facility for the Breast Cancer Task Force

Principal Investigator:	Alan Schutt
Name/Address:	Mayo Foundation
Performing Organization:	Rochester, MI

Contract Number: N01-CB-23879
Starting Date: 9/1/77 Expiration Date: 8/31/79

Goal: To establish and maintain a storage facility for serum specimens to be used by the Breast Cancer Task Force in a program designed to search for biological markers in breast cancer.

Approach: Sera specimens will be secured from breast cancer patients, benign disease patients, controls, and a screening population under separate contracts setting up a patient resource. The material will be processed, recorded and stored in −70°C deep freezers under easily retrievable conditions with all clinical data available. The sera will be used in the search for and verifiation of new breast cancer markers.

Progress: This serum bank was organized in September, 1977. Within a few months all the necessary arrangements for storage, shipping, data flow, retrieval, and quality control on serum specimens were made. The project monitor visited the three resource centers to make certain they would operate uniformly. Reports are coming in routinely. Specimens are stored at the rate anticipated.

All the specimens collected in the HIP Mammography Study were transferred from the New York Laboratory to Mayo, where they are now stored.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ihor Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $98,900 Site Visit Date: 9/28/77

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography--A Comparative Study

Principal Investigator: Name/Address	Dr. Gerald D. Dodd M. D. Anderson Hospital and Tumor Institute
Performing Organization:	Houston, TX

Contract Number: N01-CB-43993
Starting Date: 6/28/74 Expiration Date: 6/27/79

Goal: To evaluate the relative merit of conventional film mammography vs. xeromammography in the detection of breast cancer, and to delineate differences that may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among three institutions: the University of Texas System Cancer Center, M.D. Anderson Hospital, Stella and Charles Guttman Breast Diagnostic Institute and Duke University.

Mammographic image formation and informaton transmitting characteristics of each process are being assessed by way of radiographic contrast, latitude, sensitivity, and modulation transfer function. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of the work performed by the participating institutions.

Progress: Patient accrual ceased as of June 27, 1977. Preliminary statistical results indicate little difference in the diagnostic accuracy among the three modalities. Statistically significant differences have been observed in the objective physical parameters. Final results await completion of physicians' readings in order to perform fully balanced studies. Modulation transfer functions and Wiener spectral measurements of the DuPont Lo-Dose system and Kodak AA direct X-ray film were obtained for resolution and noise comparisons. At present, methods have not been established to determine the MTF and Wiener spectrum of the Xeroradiographic system in order to make a direct comparison with the Lo-Dose system and AA film. However, they have used a specially designed test object to compare resolution difference between Lo-Dose, AA film, and the Xeroradiographic system.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Date: 3/31/77

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Tissue Supply

Principal Investigator: Name/Address Performing Organization:	Paul H. O'Brien Medical University of South Carolina Charleston, SC

Contract Number: N01-CB-23867
Starting Date: 6/30/72 Expiration Date: 6/29/78

Goal: To obtain tissue and blood specimens from patients with breast cancer for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before therapy and at regular intervals thereafter. These specimens are shipped promptly to other investigators in the program for use in immunodiagnostic assays.

Progress: During the period from December 1, 1976, through December 30, 1977 the following specimens were sent: 58 preoperative blood samples from patients with malignant breast tumors, 57 malignant breast tumors, 119 preoperative blood samples from patients with benign breast tumors, 42 benign breast tumors, 23 preoperative blood samples from patients with malignant lung tumors, 21 malignant lung tumors, 106 sequential blood samples, 111 blood samples from lung cancer patients for special tests, and 218 blood samples from breast cancer patients for special tests (as requested by NIH). The blood samples were collected preoperatively, one week postoperatively, and six weeks postoperatively.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ronald Herberman, M.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Prognostic Factors for Disseminated Cancer in Patients with Early Breast Cancer

Principal Investigator:	Dr. Paul Peter Rosen
Name/Address	Memorial Hospital
Performing Organization:	New York, NY

Contract Number: N01-CB-74097

Starting Date: 8/1/77 Expiration Date: 7/3/79

Goal: To determine the frequency of subsequent metastatic breast cancer in a group of females with "early" invasive carcinoma; and to identify those clinical and/or pathologic features or groups of features that are indicators of a high or low risk for dissemination of carcinoma.

Approach: A study of population of 250 women with primary infiltrating breast carcinomas 1.0-2.0 cm in a diameter, and a subset of 100 women with malignant tumors 1 cm or less in diameter will be identified in a retrospective study (1960-1968), and followed for at least 10 years. These patients will have been treated by at least a modified radical mastectomy and have been found to have negative lymph nodes. The relationships observed between pathologic and clinical data and survival will be evaluated in order to identify those factors associated with a high or low frequency of recurrence.

Progress: All pathology records for the years 1966, 1967, 1968, and 1969 (60,617 reports) have been reviewed. Among approximately 1800 primary mammary carcinomas treated in this hospital, we have thus far identified the following numbers of cases with no axillary lymph node metastases:

A-primary 1 cm or less	110 cases
B-primary 1.2-2 cm	253 cases

Detailed analysis of pathologic features is in progress and follow-up is being sought.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.

Program: Breast Cancer Diagnosis

Technical Review Group: Breast Cancer Task Force Committee

FY 78 Funds: $73,738 Site Visit Date: 4/12/77

CONRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator:	Dr. Morton K. Schwartz
Name/Address	Memorial Sloan-Kettering Institute for Cancer Research
Performing Organization:	New York, NY

Contract Number: N01-CB-74206

Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To assay human breast cancer tissue and sera prior to masectomy and throughout the clinical course of the disease for potential markers, to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Serum and tissue specimens from patients entering for diagnosis and for primary therapy will be assayed for carcinoembryonic antigen (CEA), sialytransferase, phosphohexose isomerase (PHI) and spermine, ferritin, lactic dehydrogenase (LDH) and its isoenzymes, glucose-6-phosphate dehydrogenase (G6PD)-tissue only as a reference, β-hCG, γ-glutayltranspeptidase and α-fetoprotein and IgA. Total protein and DNA will also be determined to serve as points of reference. Sera specimens will be collected prior to surgery, before discharge and then sequentially, every three months the first year, and every six months thereafter. For those with no recurrences, specimens will be collected on a yearly basis. Tissue will be obtained at time of biopsy and mastectomy. Should recurrences follow, metastatic tissue would undergo the same testing procedure. During the first year, approximately 150-180 patients with primary breast cancer and an additional 50 patients with benign disease will enter the study. Twenty to twenty-five specimens of normal breast tissue (from autopsy specimens) will be assayed. Tissue and serum biochemical-immunochemical data will be compared and changes and differences related to the clinical status of the patient.

Progress: As of March 1, 1978, 75 patients had entered the study. Preliminary studies have been conducted to determine the concentrations of the various constituents in sections of the same tissue sample. These studies have indicated that for most constituents there is little variability. It is too early to make any statements concerning the role of these markers in following the course of patients.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $108,300 Site Visit Date: 4/12/77

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Serum LDH Isoenzymes in Women with Breast Tumors

Principal Investigator:	Dr. Ervin J. Hawrylewicz
Name/Address	Mercy Hospital and Medical Center
Performing Organization	Chicago, IL

Contract Number: N01-CB-53855

Starting Date: 6/30/75 Expiration Date: 1/31/79

Goal: To determine if a serum LDH isoenzyme pattern can be used as a diagnostic marker for breast cancer.

Approach: Serum LDH isoenzyme patterns have been determined on 800 coded patients with breast disease, benign and malignant tumors (pre- and post-operative), and from 200 coded women clinically free of breast disease (control). Based upon the single discriminator, LDH-5/LDH-1 activity ratio, isoenzyme patterns were classified as CA Type or N Type, the latter including normal, benign tumors, and breast cancer in remission (post-surgical). The diagnosis based upon the LDH isoenzyme patterns were correlated with the pathology report. In addition, one hundred samples were also correlated with the mammography report.

Progress: Initial evaluation of the data indicates that 194 serums obtained from clinically normal individuals, 80 percent were correctly categorized. Breast dysplasia, lipoma, hyperplasia, etc., at present are included into one category; of 109 patients, 75 percent were correctly diagnosed. Seventy-two percent of the 60 patients in the fibroadenoma category and 71 percent of the fibrocystic patients (122) were correctly diagnosed.

To detect recurring disease, serums from patients with previous mastectomies were studied. Eighty percent of the 73 patients without clinical evidence of recurring disease gave a normal LDH pattern. Clinical recurrence of disease was noted in 60 percent of the 32 patients.

Initial evaluations have been made on unselected pre-surgical breast carcinoma patients. Of the first group of 28 patients, the serums represented a group with clinically evident disease. Approximately 70 percent of this group were correctly identified as "Ca Type." An additional 25 serum samples were obtained at the time of mammogram examination. Of this number, only 35 percent were correctly correlated; however, 70 percent of the false negative lesions had no nodal involvement, and only 3 had evidence of metastatic disease. In contrast, 78 percent of the pre-surgical serum samples identified as "Ca Type" were from patients with positive nodes.

Increased clotting temperature increased LDH-5 activity, yielding a greater correlation in early lesions. This is being investigated.

Significance to Cancer Research: (NCP Objective 5 Appraoch 5.4)

Project Officer: Ronald Herberman, M.D., Bernice T. Radovich, Ph.D.

Program: Breast Cancer Diagnosis

Technical Review Group: Breast Cancer Task Force Committee

FY 78 Funds: 0 Site Visit Date: 10/26/76

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients with Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Per Vestergaard, M.D. Research Foundation for Mental Hygiene Orangeburg, NY

Contract Number: N01-CB-71465
Starting Date: 2/28/67 Expiration Date: 6/27/78

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross-contamination.

Progress: The special instrumentation developed under the contract has been described in a series of papers. A monograph giving full details of the methodology has been published. Data from the adrenalectomy patients studies have been published.

Significance to Cancer Research: (NCP Objective 6 Approach 6)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $10,930 Site Visit Date: 3/28/78

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator:	Dr. H. Friedell
Name/Address	St. Vincent Hospital
Performing Organization:	Worcester, MA

Contract Number: N01-CB-80704
Starting Date: 3/18/68 Expiration Date: 4/17/79

Goal: To identify and define pathologic prognostic features in cases accessioned in the Primary Therapy of Breast Cancer Study, to study these features in comparable pathologic material from British, Japanese, and Hawaiian-Japanese breast cancer patients, and to correlate this information with clinical and other laboratory data from these cases.

Approach: The pathologic material collected at the Central Pathology Laboratory from the Collaborating Clinical Centers, and that subsequently received from British, Japanese, and Hawaiian-Japanese sources, has been, and is being reviewed without knowledge of the clinical findings or other laboratory data, after which the correlations are carried out. Subsequent to the initial correlative efforts, the pathologic material is--and will be again reviewed--in an attempt to pinpoint findings of particular importance.

Progress: The tumor type and grade, nuclear grade, and presence of blood vessel invasion at the primary tumor site, and the presence of regional lymph node involvement has been determined in 381 cases, and a report defining the study has been published (JNCI 53: 603-607, 1974). A report of some clinicopathologic correlations was presented at the May, 1977 ASCO meeting in Denver, and a manuscript has been submitted for publication. A workshop on sinus histiocytosis, using material from the Central Pathology Laboratory, was carried out, and a report has been submitted for publication. A review of sinus histiocytosis the 381 cases is in progress. A joint review with other pathologists of sinus histiocytosis in axillary lymph nodes from Japanese, Hawaiian-Japanese, and British breast cancer patients has been completed, and a manuscript is in preparation. A detailed review of all axillary lymph nodes in radical masectomy specimens from Japanese patients treated five or more years at the National Cancer Center Hospital in Tokyo is almost completed.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/25/70

CONTRACT RESEARCH SUMMARY

Title: Prognostic Significance in Breast Cancer of Regional Lymph Node Immune Response

Principal Investigator: Name/Address Performing Organization:	Dr. Susanna Cunningham-Rundles Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number:
Starting Date: Expiration Date:

Goal: Evaluation of the relevance of regional lymph node immune response to prognosis in breast cancer. This will require an assessment of cell-mediated immune reactivity in well-standardized *in vitro* assays to defined antigens associated with the development of disease.

Approach: Immunological procedures will be performed on lymph nodes of 100 patients with primary operable breast cancer, using a total of 4-6 lymph nodes per patient including both negative and positive nodes, ideally from each level (I-III). In addition, control lymph nodes will be obtained from 50 other patients. The assays to be done on both peripheral blood and lymph node mononuclear cells include leukocyte migration inhibition factor (LMIF) and lymphocyte transformation tests, the latter comprising PHA, Candida, *E. coli*, T-antigen and MuMTV. A third class of newer tests includes natural killer cells, suppressor cells, cell surface markers and monocyte function assays. Clinical and demographic data as well as histopathologic data on tumor size and histologic type will be obtained. Three-year follow-up information on recurrence and survival status will be recorded. Correlation of all factors will be statistically performed.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $100,000 Site Visit Date: 4/27/78

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid (BCF)

Principal Investigator: Dr. Morton K. Schwartz
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: N01-CB-53853
Starting Date: 6/30/75 Expiration Date: 9/14/79

Goal: To establish the biochemical and immunochemical composition of human BCF; to search for a marker which might be useful in understanding the formation of cysts, their treatment, and the risk of patients with cysts developing cancer.

Approach: BCF from patients with cystic mastopathy will be analyzed for polypeptide and steroid hormones, tumor associated antigens, enzymes, lipids, minerals, and trace elements. These data will be correlated with each other and with the demographic information. Fluid will be analyzed from patients with recurrent cysts, and clinical follow-up will be obtained of all patients.

Progress: As of March 1, 1978, 940 patients have entered the study. Thirty of these patients have breast cancer. About 25% of the patients have had recurrent cysts and additional specimens submitted for analysis since their initial aspiration. As previously noted, the concentrations in many BCF specimens of CEA, α-fetoprotein, β-subunit hCG, calcium, copper, zinc, total protein, albumin, cholesterol, phosphohexose isomerase, γ-glutamyltranspeptidase, β-glucuronidase, lactic dehydrogenase, amylase, dehydroisoandrosterone, androsterone, and their sulfates are many fold the concentrations of these constituents found in serum. Correlation of BCF concentrations for many of these constituents in the same patients were found to be significant. The specimen variation was much less in specimens from the same patients obtained either at the same time or on subsequent aspiration than that from patient to patient. Notwithstanding the large BCF to serum gradient, isotope studies have indicated a to contain material both of a secretory nature and that resulting from cell breakdown. BCF contains low but detectable levels of terminal deoxynucleotidyl transferase (TdT) and DNA polymerases were observed in BCF and in supernatant from cells from BCF maintained in culture media. Patient accrual will cease as of September 14, 1978, with five year follow-up planned.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds $10,000 (approximately) Site Visit Date: 5/5/77

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Dr. Howard H. Sussman
Name/Address Stanford University Medical Center
Performing Organization: Stanford, CA

Contract Number: N01-CB-74086
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To assay human breast cancer tissue and sera, prior to masectomy and throughout the clinical course of the disease, for potential markers to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Tumor and sera of patients presenting initially for diagnosis and primary therapy (Category 1) will be assayed for the fetoplacental proteins; i.e., placental alkaline phosphatase (PAP), human chorionic gondotropin (hCG) α and β, human placental lactogen (hPL), and carcinoembryonic antigen (CEA), and tests on sera will be repeated sequentially every three months. Patients developing recurrences (Categories 2 and 3) will be included with a study population from the Northern California Oncology Group. Another objective of the study will be to identify new ecotopically synthesized placental membrene proteins, which may be common to both trophoblasts and neoplastic breast tumor cells.

Progress: Twenty patients from Category 1 have been tested thus far for tumor markers, and initial, pre-operative specimens indicate elevated levels of serum PAP (<2 ng/ml) in 10% of the patients, and α-hCG (>3 ng/ml) in 5%. The elevated levels of the markers were beyond the range of a group of post-menopausal controls. The β-hCG, hPL, and CEA assays have not yet been completed.

Fifty-two patients from Category 3, being treated for metastatic disease, have been tested for serum PAP and α-hCG. PaAP was elevated in 26% of these patients, and β-hCG was elevated 14%. The levels of PAP and α-hCG in positive patients were generally higher in the Category 3 patients than in Category 1 patients positive for the same markers.

Three syncytial trophoblast brush border membrane glycoproteins have been characterized with respect to their subunit M_r and pI, and reaction with antisera raised against the placental brush border membrane glycoproteins. They appear to be placental specific with respect to their absence from liver and kidney membranes. These components may be useful as additional tumor markers.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $117,500 Site Visit Date: 4/4/77

CONTRACT RESEARCH SUMMARY

Title: Detection of Immune Complexes in Sera of Patients with Breast Cancer

Principal Investigator:	Dr. M. Edward Medof
Name/Address	University of Chicago
Performing Organization:	Chicago, IL

Contract Number:

Starting Date: 9/1/78 Expiration Date: 8/30/79

Goal: To assay sera from patients with breast cancer, benign breast disease and normal volunteers for immune complexes (IC) employing multiple techniques for IC detection and quantitation, then to correlate presence or absence, or levels where applicable, of individual IC subpopulations with tumor progression, response to therapeutic intervention and clinical course.

Approach: Four detection techniques for ICs (Raji-cell receptor assay, conglutinin-binding assay, solid phase Clq binding assay, and PEG-Clq precipitation assay) are established in this laboratory and will be run on sera of 50 new breast cancers, 250 benign disease patients, 84 patients with disseminated disease and 600 normals in the first year. Sera and plasma samples will be assayed for soluble and insoluble, complement bearing and IgG-Fc accessible ICs. Data will be analyzed according to clinical stage of patients, histologic type of tumor, histology of regional lymph nodes, ovarian and other hormonal status of patient and the presence or absence of estrogen receptors on tumor cells.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.

Program: Breast Cancer Diagnosis

Technical Review Group: Breast Cancer Task Force Committee

FY 78 Funds: $46,300 Site Visit Date: 4/26/78

CONTRACT RESEARCH SUMMARY

Title: Mass Screening for Breast Cancer by Electronic Infrared Pattern Recognition

Principal Investigator: Name/Address Performing Organization:	Dr. JoAnn D. Habermann University of Oklahoma Health Science Center Oklahoma City, OK

Contract Number: N01-CB-43869
Starting Date: 4/1/74 Expiration Date: 9/29/79

Goal: To design and clinically test a prototype system for mass screening for breast cancer using quantitative thermography and computer processing with automated methods of evaluation.

Approach: This project is Phase II of a proposed 3-phase development Program. Phase I included: 1) Construction of an improved thermographic instrument designed with the unique serial scan multiple element detector developed by Honeywell. This instrument provides radiometrically calibrated infrared imagery that is subsequently digitized. It is equipped for recording on a standard video recorder and for conventional television display. 2) Initiation of a program of clinical evaluation to assess the utility of various quantitative parameters in selecting the thermographic changes of breast cancer. 3) A time-series evaluation of a group of subjects to obtain estimates of the influence of several physiological factors on the breast thermal patterns. Phase II consists of further analysis of a variety of pattern recognition schemes for feature development and classifier design and construction.

Progress: Clinical evaluations are proceeding on groups of symptomatic and asymptomatic subjects. The early months of Phase II were devoted to system improvement with the addition of a 6 Bit A/D conversion video digitizer, which provides "on-line" digitization of the thermal image. Presently, all thermograms are digitized "on-line." During the period of system modification, absolute temperature measurements on 871 patients were manually recorded including 50 cancers. To date, 10,084 examinations (including 73 cancers) are included in the post-on-line digitization data base. Breast temperature measurements are being examined to determine their ability to discriminate between cancer and control subjects. Forty-four quantitative thermal feature parameters have been identified and are being examined as possible diagnostic indicators. The blood perfusion estimate statistic appears to be one of our best discriminators. A total of 150 cancers will be included in the data base of Phase II of this program.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $100,000 (approximately) Site Visit Date 4/1/77

CONTRACT RESEARCH SUMMARY

Title: Risk Associated with In-Situ Carcinoma and "Precancerous" Mammary Hyperplasias

Principal Investigator: Dr. David L. Page
Name/Address Vanderbilt University School of Medicine
Performing Organization: Nashville, TN

Contract Number: N01-CB-74098
Starting Date: 7/15/77 Expiration Date: 7/14/79

Goal: To compare subgroups of females regarding their incidence of breast carcinoma during a 10 to 25 year interval after biopsy diagnosis of *in situ* carcinoma and mammary hyperplasias.

Approach: This will be a retrospective study in which slides will be reviewed and classified on over 4000 patients who had biopsies performed during 1952-1969. This study population will then be contracted and follow-up information on each subject obtained regarding subsequent development of mammary carcinoma, as well as other pertinent clinical and epidemiological data. The control group will be composed of patients with biopsy but no evidence of proliferative lesions. An effort will be made to identify those specific breast lesions or combinations of lesions which are associated with an increased risk of development of breast cancer. Non-anatomic risk factors will also be correlated.

Progress: 900 contact letters have been mailed with over 500 responses. 320 completed questionnaires are filed. Surgical Pathology logbooks from three hospitals have been completely reviewed, 1952-1968, creating a list of all breast operations. A data quality assurance program has been designed and implemented. Histologic classification is complete for 1150 biopsies. 18 cases of *in situ* carcinoma are identified.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radivich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $104,100 Site Visit Date: 4/13/77

CONTRACT RESEARCH SUMMARY

Title: Biologic Markers in Breast Cancer: Patient Resource

Principal Investigator:	Dr. Leslie W. Whitney
Name/Address	Wilmington Medical Center
Performing Organization:	Wilmington, DE

Contract Number: N01-CB-74137
Starting Date: 9/1/77 Expiration Date: 8/31/79

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls to be used in a search for, and verification of, new breast cancer markers.

Approach: 30 cc of blood will be collected from breast disease patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions prior to surgery. Another specimen will be collected, when feasible, 510 days post-masectomy from the same patient. Serum specimens will be stored at -70°C then shipped to NCI-designated blood bank facility with appropriate clinical data.

Progress: This study actually began on December 29, 1977. As of March 31, 1978, seventeen patients have been entered into the study, twelve with benign breast disease, and five patients with malignant breast disease. A total of 153 vials have been shipped to the Mayo blood bank. As required, patients with malignant disease must also consent to a second specimen postsurgically and a third specimen on an annual basis. The biological markers clinical data forms are completed and forwarded by separate mail to the Mayo Clinic.

Significance to Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Ihor Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $28,150 Site Visit Date: 5/16/78

CONTRACT RESEARCH SUMMARY

Title: Estrogen Replacement after Pre-Menopausal Oophorectomy and Breast Cancer Risk

Principal Investigators: Drs. Dennis Sloan and Samuel Shapiro
Name/Address: Boston University Medical Center
Performing Organization: Cambridge, MA

Contract Number: N01-CB-74099
Starting Date: 9/1/77 Expiration Date: 8/31/78

Goal: To examine the hypothesis that exposure to female hormones, particularly estrogen replacement therapy, is related to an increased risk of breast cancer, either during use or after a latent interval.

Approach: An epidemiological study of the case-control type is being implemented as part of an ongoing, hospital-based, multicenter data collection system. Cases of breast cancer and 2 control groups are interviewed by trained nurse-monitors in hospitals throughout the country. One control group consists of women with cancers not suspected to be related to female endocrine physiology. The second group of controls comprises hospitalized women with a variety of benign conditions. Information on lifetime drug use and relevant medical data are recorded on standard forms and transferred to computer files for analysis.

Progress: As of the end of March 1978, 190 women with breast cancer had been studied. Of these, 60% were newly diagnosed and 20% were menopausal because of bilateral oophorectomy. Women with breast cancer are currently being interviewed at a rate of 480 per year, of whom about 50 will be incident cases who are menopausal because of bilateral oophorectomy.

In our data, rates of conjugated estrogen use among potential controls are 3% among premenopausal women, 15% among naturally post-menopausal women, 20% among women menopausal becuase of hysterectomy who retained at least one ovary, and 39% among women menopausal because of bilateral oophorectomy.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Robert Hoover, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $39,550 Site Visist Date: 2/25/77

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator:	Dr. Baruch Modan
Name/Address	Chaim Sheba Medical Center
Performing Organization:	Tel Hashomer, Israel

Contract Number: N01-CB-74102
Starting Date: 8/1/77 Expiration Date: 8/31/78

Goal: To determine the characteristics of patients with different types of non-invasive breast lesions, with particular reference to diet and to those factors reputed to be preferentially associated with breast cancer, and to compare the findings with our similarly conducted breast cancer study.

Approach: The study consists of two phases: (a) case-control interview study of 600 cases of non-invasive breast diseases in terms of such risk factors as: dietary habits, age at first birth, menstrual history, menopausal status, parity, height and weight, familial breast cancer, selected drug history, and previous radiation exposure; and (b) an incidence study of non-invasive breast lesions in a total community. The characterization of the breast lesions will take account of the histological pattern, degree of atypia, and lymphoreticuloendothelial response (LRE). Particular attention will be paid to the relative frequency of distinct histo-pathological forms in high and low risk subpopulations (i.e. Europeans, Asian-African born).

Progress: Procedures have been developed for identifying benign breast disease patients and controls, most of whom have a short hospital stay. As of 3/31/78, home interviews have been conducted on 149 cases, 56 minor or short stay surgery controls, and 74 neighborhood controls. Controls are identified after the case home interview is complete, and they are interviewed approximately two months after their matched case. The questionnaire is the same as that being used in our breast cancer study. These cases represent the first 149 consecutive cases, to see the ethnic and pathology distributions; subsequent cases will be selected to fill the required distribution of ethnic origin and pathological classification. Original pathologic diagnosis is taken from the hospital's frozen section department. Slides immediately following those used for this diagnosis are then given to the study pathologists for review and reclassification by the convention histological methods by Dr. Black's grading system for atypia. The first 149 cases represent a mix of all ethnic groups, but two-thirds of the cases over age 50 are of European or American origin.

Significance for Cancer Research: (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D., William Blot, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $82,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. Henry T. Lynch
Name/Address: Creighton University School of Medicine
Performing Organization: Omaha, NE

Contract Number: N01-CB-44003
Starting Date: 6/17/74 Expiration Date: 6/18/78

Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high- and low- risk breast cancer families.

Approach: HL-A markers, genetic polymorphic markers such as red blood cell enzymes (acid phosphatase, adenosine deaminase, etc.), serum proteins (haptoglobins a-chain, group-specific component, etc.), and blood group systems (ABO, Duffy, Lewis, MN, etc.), and specific pathologic diagnoses (all histologic varieties of cancer retrospectively in the family context) are being evaluated in approximately 20 multiple cancer case families selected from the total clinical pool of high-risk families and kindreds. Segregation and linkage analyses of pedigrees will be performed, and quantitative biomarkers will be incorporated into an index of breast cancer susceptibility using general methods for deriving selection indices.

Progress: Twenty-one case families have been sampled. A total of 742 individuals have been seen and blood samples obtained. Specimens have been sent to the investigators. HL-A data have been analyzed for seven of the breast cancer-prone families, and other markers have been analyzed for nine families categorized as at highest risk. Linkage analysis is now being undertaken. Eighty-five pedigrees appear suitable for segregation analysis, and 18 of these will be subjected to linkage analysis contingent upon confirmation of qualitative inheritance. The resource continues to show genetic heterogeneity as evidenced by at least five distinct tumor entities. Twelve pedigrees show predisposition to breast/ovarian carcinomas with an approximate 50% lifetime risk to daughters of affected mothers. An even higher rate of transmission from affected paternal grandmothers leaves open the possibility of X-linkage in these particular pedigrees.

Significance for Cancer Research: (NCP Objective 5 Approach 5.14)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 2/3/77

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. David C. Deubner Duke University Medical Center Durham, NC

Contract Number: N01-CB-63996
Starting Date: 6/29/76 Expiration Date: 6/29/78

Goal: The goal is to define the epidemiologic characteristics of breast cancer in pre- and postmenopausal women and to relate these characteristics to the pathobiology of the tumor as manifested by estradiol and progesterone receptors, histology, and cellular ultrastructure.

Approach: This case-referent study is of breast cancer patients undergoing surgery at university and community hospitals, surgical or medical patients not having breast or gynecological surgery at the same hospitals, and subjects from the community. Data collection is by interview of all subjects and review of charts of hospital patients. Laboratory studies on tissue and serum from breast cancer patients include quantitative estrogen and progesterone receptor determinations, evaluation of histology and ultrastructure by a standardized protocol, and estrogen and progesterone serum determinations. Statistical analyses will estimate the relative strength of association between breast cancer in pre- and postmenopausal women and such factors as age of menarche, age at first delivery, obesity, and family history of breast cancer. Associations among risk factors, tumor cell biology, and morphology will be sought by comparing the laboratory results in women with and without single or multiple risk factors.

Progress: Effective working relations have been established with the clinicians and pathologists in the three participating hospitals. As of August 31, 1977, 237 women with breast cancer had been entered in the study, and satisfactory interviews and pathologic materials were obtained from at least 85%. Two hospital referent subjects, matched by race and age, have been interviewed for each case entered. The community referent group is a random sample of women at least 30 years of age who live in the Piedmont and Plains regions of North Carolina. Approximately 700 of the anticipated 1,800 interviews have been completed since the field work began in March 1977. Laboratory procedures are being completed according to protocol, as material is obtained from the participating pathologists.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Gordon B. Cutler, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $170,000 Site Visit Date: 5/5/76

CONTRACT RESEARCH SUMMARY

Title: Endocrine Events at the Time of First Pregnancy

Principal Investigator: Dr. John R. K. Preedy
Name/Address Emory University School of Medicine
Performing Organization: Atlanta, GA

Contract Number: N01-CB-74101
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: It is proposed that the protective effect of an early first pregnancy against breast cancer is due to a change in the hormonal environment following that event. We proposed to investigate and characterize such a change.

Approach: Nulliparous subjects in the 18-22 age range who have not taken birth control pills, who have a history of normal regular menses, and who are planning to become pregnant in the next 1-2 years will be selected (Group A). Similar subjects will be chosen who do not plan to become pregnant in the next 1-2 years (Group B). Similar subjects to the above but in the age range 30-40 will also be selected (Groups C and D). All groups will have the following carried out in the early follicular phase of the menstrual cycle: plasma gonadotrophins, estrogens, progesterone, androgens, urine estrogens and androgens, plasma protein steroid binding studies, perphenazine-prolactin stimulation tests; and LRH-LH stimulation test. Results from Groups A, B, C, and D will be compared by appropriate analysis of variance techniques to determine significant contrasts between the four groups.

Progress: Due to difficulty encountered in recruiting subjects who are planning pregnancy within the next 1-2 years, criteria for subject selection have been modified to include subjects who have had their first and only full term pregnancy within the last 1-3 years, but who otherwise satisfy our other criteria. Perphenazine has been substituted for chlorpromazine for prolactin stimulation, due to the marked postural hypotensive effect and drowsiness caused by chlorpromazine. The protocol has also been modified to include that which does not involve prolactin and LH stimulation tests, since some subjects have indicated they did not want any medications. About 40 subjects have been recruited so far: 13 subjects have been tested, 25 are waiting to be tested within the next two months.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Richard Knazek, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $121,500 Site Visit Date: 6/6/77

CONTRACT RESEARCH SUMMARY

Title: Pathogenetic Models of Malignant and Premalignant Disease of the Breast

Principal Investigator:	Drs. J. A. H. Lee and S. H. Moolgavkar
Name/Address	Fred Hutchinson Cancer Research Center
Performing Organization:	Seattle, WA

Contract Number: N01-CB-74100
Starting Date: 9/1/77 Expiration Date: 8/31/78

Goal: To translate hypotheses, such as the multistage theory, about the development of breast cancer into mathematical terms, and to test the predictions of these against a range of age-specific incidence rates of the disease.

Approach: The behavior of the incidence curves generated by models will be studied under perturbations of the parameters. Maximum likelihood estimations will be used to fit the model to population data that have been statistically analyzed to allow for the effects of secular trends on age distribution.

Progress: A paper on the age distribution of breast cancer in males in Japan and the U.S. and another on the theoretical discussion of the multistage theory of carcinogenesis and its implications for cancer incidence curves in humans are to appear in JNCI. Female breast cancer incidence data from Iceland, Osaka, Connecticut, and Denmark, and mortality data from the U.S., England and Wales, Canada, and Japan have been analyzed. The single most important finding is that after adjustment for secular trends, all the incidence curves have very similar shapes. In particular, the adjusted incidence curve in Osaka continues to rise after menopause. The fall in rate after menopause observed in cross-section data is due to the operation of a strong generation effect. This work is currently being written up for publication.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Sylvan Green, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $83,678 Site Visit Date: 3/25/77

CONTRACT RESEARCH SUMMARY

Title: Lipid and Cholesterol Levels in Relation to Human Breast Cancer Risk

Principal Investigator:	Dr. Gary D. Friedman
Name/Address	Kaiser Foundation Research Institute
Performing Organization:	Oakland, CA

Contract Number: N01-CB-84238
Starting Date: Expiration Date:

Goal: To explore a possible etiologic role of dietary fat in breast cancer, by examination of breast cancer incidence in relation to previous levels of cholesterol and/or lipids.

Approach: We plan, specifically, to compare incidence rates of breast cancer and associated cancers in groups of women defined by their serum cholesterol and/or lipid levels at a time prior to their diagnosis. Using the resource of data from the Northern California Kaiser Health Plan's multiphasic health check-ups (95,000 women between 1964 and 1976), we estimate that more than 250 cases of breast cancer will be available for review. Medical data on cholesterol and, for some, on lipid levels will be available. Other known and available risk factors and possible confounding variables, such as obesity and ethnic background, will be considered in analysis.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $196,000

CONTRACT RESEARCH SUMMARY

Title: Relationship Between Thyroid Diseases and Breast Cancer

Principal Investigator: Dr. Farahe Maloof
Name/Address Massachusetts General Hospital
Performing Organization: Boston, MA

Contract Number: N01-CB-84230
Starting Date: Expiration Date:

Goal: To examine the possible association of breast cancer with prior thyroid dysfunction.

Approach: This will be a retrospective cohort study designed to measure the rates of breast cancer in women with and without prior thyroid disease. A mortality study will follow 7,000 women with thyroid disease, seen at the Thyroid Clinic of the Hospital between 1921 and the present, and 7,000 matched population controls; 2,000 women seen at the Clinic but without identified thyroid disease will also be followed. Rates will be compared in women with various types of thyroid disease. A morbidity study will follow 1,600 women with previous thyrotoxicosis; this will include 500 women who became hypothyroid following the treatment and 500 who did not. Rates of breast disease will be compared according to mode of treatment as well as externally compared with rates from the Connecticut Tumor Registry.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.1.4)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $173,000 Site Visit Date: 2/22/78

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator:	Dr. H. Stephen Gallager
Name/Address	M.D. Anderson Hospital and Tumor Institute
Performing Organization:	Houston, TX

Contract Number: N01-CB-53854
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To demonstrate morphologic markers characteristic of familial types of breast disease in order to identify selected high-risk populations.

Approach: A previously developed system for the profiling of the morphologic characteristics of breast cancer which integrates information from gross and microscopic examination, mammography, and specimen radiography and requires the rough quantitative evaluation of some 39 factors in three groups to be validated and refined. The breast cancer specimens will be derived from three major risk groups which have been characterized genetically. These consist of 114 patients with mothers with breast cancer, 97 patients with sisters with breast cancer, and 111 patients with second order pedigrees for breast cancer. The findings will be compared with 300 patients without family histories.

Progress: The planned validation phase of the study has been completed, and modifications of the data collection system and evaluative criteria have been completed. A paper is in preparation concerning the biomathematical techniques used in this phase. It is believed that they may have relevance to the development of quality control procedures for diagnostic histopathology. General data recording using the refined system began about December 1, 1975. To date, approximately 235 cases have been completed by all three observers, and an additional 20 cases have been completed by one or two observers. Preliminary analysis based on partial data shows no significant differences among the groups other than a slight tendency for lymphoid infiltration in carcinomas in the familial groups.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Philip Prorok, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 1/28/76

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Name/Address Performing Organization:	Dr. Frans J. Cleton The Netherlands Cancer Institute Amsterdam, The Netherlands

Contract Number: N01-CB-43940
Starting Date: 6/18/74 Expiration Date: 6/18/78

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high-risk groups in the population for the development of breast cancer.

Approach: HL-A haplotypes will be studied in families with a high incidence of breast cancer. Certain genetic markers located on the 6th chromosome and the red blood cell groups will also be investigated. The study of red cell isoenzymes, immunoglobulins, and chromosomal polymorphisms have been stopped after the analysis of data on 14 families. The search for cross-reacting antigens with mouse MTV-sera, yielding negative results, has also been stopped. Plasma-prolactin levels will be measured in breast cancer patients and their daughters, with special emphasis on diurnal fluctuation and variation during the menstrual cycle. Because multiple case families with sufficient living affected members in two generations are rare, it was decided also to investigate pairs of sisters with breast cancer. Linkage of breast cancer with HL-A can be determined by comparing similarities in HL-A haplotypes in these pairs with the expected similarity.

Progress: Linkage studies have been carried out on 700 members from 14 high-risk families, including 60 patients with breast cancer. There was no significant evidence of linkage between breast cancer and any of the 70 genetic markers that were studied. A computer program for linkage analysis, especially designed for incomplete family data, was used, and the results were expressed as LOD scores. Because the sample may have been too small for this method of analysis, more high-risk families will be investigated. New methods for analyzing the results of the family studies will be used. The sample will be increased with similar data from the investigation of two other contractors. Ten sister pairs have been typed for HL-A in addition to the family studies. Studies of prolactin in healthy female relatives in these families showed an irregular distribution of plasma levels, probably related to an altered diurnal regulation. These studies will be extended, including the variation of plasma prolactin during the menstrual cycle.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Interrelationships Among Diet, Steroid Hormone Metabolism, and Breast Cancer Risk

Principal Investigator: Dr. Marvin A. Kirschner
Name/Address Newark Beth Israel Medical Center
Performing Organization: Newark, NJ

Contract Number: N01-CB-84229
Starting Date: Expiration Date:

Goal: To investigate steroid hormone metabolism in relation to obesity and dietary patterns.

Approach: This will be an in-depth study of steroid hormone production, metabolism, and excretion in a cohort of obese American women undergoing drastic weight reduction and diet manipulation. Forty obese, but otherwise healthy and normally menstruating women will be studied prior to weight reduction, after weight loss and stabilization on a low carbohydrate, low fat diet, and after isocaloric exchange to a more typical high carbohydrate, high fat American diet. The biochemical studies will examine androgen, estrogen, and cortisol production rates, interconversion, and peripheral metabolism. Values will be compared under the three sets of conditions and further compared with those in lean, age-matched controls.

Progress:

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $188,000 Site Visit Date: 3/17/78

CONTRACT RESEARCH SUMMARY

Title: Relation of Intestinal Flora to Breast Cancer in Humans

Principal Investigator: Dr. Sherwood Gorbach
Name/Address: New England Medical Center
Performing Organization: Boston, MA

Contract Number: N01-CB-74104
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To determine whether diet and other risk factors influence the metabolism of estrogens by bacteria in the large bowel.

Approach: The estrogen profiles will be determined in the serum, urine, and feces of young and older women eating "Western" or vegetarian diets, and in women with a history of breast cancer. The ability of the fecal flora to metabolize estrogens will also be studied. An analysis of fecal metabolic activity will be done for the different experimental groups. Novel fecal metabolic transformations of estrogens and the possible role of these transformations in the etiology of breast cancer will be investigated.

Progress: In the first year 33 women have been enrolled in this study. Between 6 and 8 women in each of the dietary and age groups are now participating in the study. In addition, four breast cancer patients are providing specimens. A medical and dietary history has been taken on each subject. Standard clinical chemistry tests have also been performed. A 72-hour urine, feces, and blood sample has been collected from the 33 subjects. More than half of the subjects have now provided a second series of specimens (collected period 2). A total urine volume and creatinine concentration has been measured. The total fecal weight percentage of water content, and concentration of fecal protein have also been determined. The urinary levels of estrone, estradiol, estriol, estriol-3-glucuronide, and estriol-16-glucronide have been measured for 28 subjects. The concentration of fecal estrone, estradiol, and estriol have also been measured for 28 subjects. Serum estrone, estradiol, testosterone, and androstenione-Δ^4dehydragenose activities have been assayed. The formation of enzymatic products has been measured by gas-liquid chromatography. More than 70 enzymatic assays have been completed. Computer analysis of the dietary histories revealed a significant difference in the intake of total carbohydrate, animal protein, and animal fat between the vegetarian and omnivore groups. This analysis clearly indicates we have selected vegetarians and omnivores for this study.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Carl Smith, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $102,500 Site Visit Date: 4/13/77

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Minimal Breast Cancer in Initially Asymptomatic Women

Principal Investigator:	Dr. Bernard Pasternack
Name/Address	New York University Medical Center
Performing Organization:	New York, NY

Contract Number: N01-CB-74103
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: Investigate by means of risk factor analysis whether minimal breast cancer in initially asymptomatic women is essentially different from clinical breast cancer or whether it is an early manifestation of the same disease; identify risk factors associated with tumor aggressiveness.

Approach: We will obtain and analyze demographic and medical data recorded at the Guttman Breast Diagnostic Institute, which since 1968 has been offering free breast examinations to women living in New York City. The study group will consist of an estimated 880 valid cases detected through the end of 1978 and a random selection of approximately 2,700 controls. Two independent histopathologic diagnoses will be performed. Cases will be compared according to three tumor criteria: a) level of invasiveness, b) level of aggressiveness (prevalent vs. incident cases), and c) histological type. Controls will be used to confirm relative risk estimates for study factors. Comparison of all cases to controls should result in estimates of relative risk within the range found at other screening centers, whereas the comparison of clinical cases to controls should provide estimates within the same order of magnitude as registry data.

Progress: Protocols have been established for all phases of the study: collection of demographic and screening data, pathology review, and mammography review. Basic identification forms have been coded and keypunched for all 1,146 cases diagnosed through October 1977. Examination code forms for about 470 participants have been coded, and of these, about 280 have been keypunched. Initial and recall histories have been coded for about 350 participants, and of these, about 160 have been keypunched. A preliminary computer program for editing the six data forms has been developed. In addition, routing logs, which monitor progress in the various phases of the study for each participant, have been computer-generated.

Significance for Cancer Research: (NCP Objective 5 Approach 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D., Benjamin Hankey, Sc.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $112,400 Site Visit Date: 2/17/77

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Medullary and Lobular Breast Cancer

Principal Investigator: Dr. Paul P. Rosen
Name/Address
Performing Organization: Sloan-Kettering Institute for Cancer Research
New York, NY

Contract Number: NO1-CB-63997
Starting Date: 6/29/76
Expiration Date: 6/29/78

Goal: To identify and describe morphologic features of human female mammary carcinoma which are significantly related to known risk factors for the development of such carcinomas.

Approach: Detailed epidemiologic information will be obtained from interviews with women with mammary carcinoma and correlated with pathologic features of the tumors. The analysis of the data will emphasize comparison of medullary and lobular carcinoma with duct carcinoma, but will also permit the study of morphologic features not limited to one tumor type.

Progress: Four hundred and ninety-seven consecutively treated patients have been entered into the study. Among cases already reviewed are 31 women whose only lesion was medullary carcinoma, 19 with only in situ lobular carcinoma, and 28 with only infiltrating lobular carcinoma. In addition, 70 patients had lobular carcinoma (22 invasive, 48 non-invasive) combined with another type of carcinoma, and 9 patients had medullary carcinoma associated with another type of carcinoma. Interviews have been obtained with at least 97% of the patients. Data verification is being carried out for the information obtained by interview which related to prior medical or surgical therapy. Three hundred complete, verified records have been entered for computer storage.

Significance for Cancer Research: (NCP Objective 5 Approach 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D., Roger Connelly
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $85,000
Site Visit Date: 4/21/76

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator:	Dr. Bjorn Stenkvist
Name/Address	University Hospital
Performing Organization:	Uppsala, Sweden

Contract Number: N01-CB-53968
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications, and established epidemiological risk factors of breast cancer.

Approach: Within a clearly defined area and population (4 Swedish countries) 181 breast cancer cases occurred during a defined study period (5 months). From 179 of these patients, a number of epidemiological data could be obtained as well as morphological data. From 179 exactly age-matched controls, epidemiologic data were recorded in the same way as the patient data. The tumors were also characterized by computerized photometry of the cell populations in the tumors in addition to conventional morphology. In the first phase of the epidemiological study, breast cancer was regarded as an entity (i.e., not morphologically subdivided), and the patients were compared with their exactly age-matched controls.

Progress: The comparison between patients and controls showed no significant difference in age at menarche, age at first birth, age at menopause, number of children, height, weight, obesity, or frequency of diabetes and hypertension. These results are at variance with most earlier reports, possibly because the controls here were selected from the whole female population instead of hospitalized patients. Heredity and frequency of earlier breast disease were significantly higher in the patient group. We have also developed a new basis for division of tumor cell populations from breast cancers, based upon photometry and digital picture analysis. By this method, a measure of atypia has been obtained for each tumor. The computed measure is positively correlated to other biological parameters such as mitotic rate in the tumor. It also has a significantly higher reproducibility than subjective systems for classification or grading of breast cancer.

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Elizabeth P. Anderson, Ph.D., Cecil Fox, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 1/17/75

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Ralph S. Paffenbarger University of California Berkeley, CA

Contract Number: N01-CB-63998
Starting Date: 6/29/76 Expiration Date: 6/29/78

Goal: This work would identify host and environmental characteristics that predispose to the development of breast cancer in women of all ages, and would contrast the findings between premenopausal and postmenopausal women.

Approach: Using epidemiologic methods of case-control design, we would conduct structured interviews with women in the San Francisco Bay area, totaling over 2,500 breast cancer patients and 5,000 control women. Characteristics to receive special.attention as potential precursors of breast cancer would include age, catamenial and childbearing events, indicators of early fertility, exogenous hormone and drug use, sociocultural factors, family history of cancer, radiation exposure, and various physical measurements. Extent of disease (staging), histological diagnoses, and survival determinations would be established. Amassed data would be processed using appropriate statistical methods in search for associations between epidemiologic characteristics and breast cancer, premenopausal vs. postmenopausal.

Progress: Interviews have been completed on some 4,000 study subjects, one-third of whom are breast cancer patients of all ages and two-thirds of whom are age-, race-, and hospital-matched control patients. About 20% of the breast cancer patients are premenopausal. Nearly 3,900 interviews have been coded and keypunched for data processing. A series of computer-based editing filters has been programmed to check the validity and consistency of the data. Procedures to re-code and update edited records have been instituted; editing is about two-thirds complete on 2,400 subjects. A general computer program has been constructed to perform summary chi square anlayses with adjustment for confounding factors, starting from primary data and user-prepared control cards. Documentation of the program has been prepared. Histological diagnoses have been made on about 500 breast cancer patients; these data are being coded and keypunched. Survival followup of breast cancer patients has been completed through December 1976. Preliminary analyses have been made of the risk of breast cancer as it depends upon overuse of oral contraceptives, total duration of oral contraceptive use, and use of oral contraceptives before first childbirth.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1 and 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D., Bruce Stadel, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $80,000 Site Visit Date: 4/15/78

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator:	Dr. Gary Spivey
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-74202
Starting Date: 8/15/77 Expiration Date: 8/14/78

Goal: To examine the epidemiology of major types of benign breast disease and to compare identifiable risk factors for benign diseases with known risk factors for breast cancer. To examine a histologic classification scheme based on grading of cellular atypia in comparison to the conventional histologic classification.

Approach: Women with first biopsies for benign breast disease in participating hospitals will be admitted to the study by a sampling mechanism designed to represent the spectrum of histologic types. A subcategory of women with breast cancer will be included. Two types of controls will be used, a hospital control and a friend control. Information on epidemiologic characteristics will be obtained by questionnaire. Epidemiologic characteristics of different histologic types of benign breast disease will be compared to each other and to those of breast cancer.

Progress: Participation has been enlisted from seven hospitals doing breast biopsies in the Coastal Health Services Region, and procedures have been established for identification of cases and of hospital controls. A pilot population study has determined the distribution of age and race and of benign breast disease diagnoses, and the sampling plan will soon be completed. Pathology slides will be classified according to both traditional pathologic classification and the Black-Chabon grading of cellular atypia. A questionnaire has been developed and pretested. It seeks responses on a wide range of topics including environmental influences, familial cancer patterns, medical, maturation, reproduction history, and various other endocrine-related subjects. Interviewer training has begun, but interviewing has had to be delayed by the necessity for OMB clearance of the questionnaire.

Significance for Cancer Research: (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D., Richard Knazek, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Force Committee
FY 78 Funds: $121,500 Site Visit Date: 6/6/77

CONTRACT RESEARCH SUMMARY

Title: Role of Dietary Factors and Non-Contraceptive Estrogens in Breast Cancer

Principal Investigator:	Dr. Abraham Nomura
Name/Address	University of Hawaii at Manoa
Performing Organization:	Cancer Center of Hawaii Honolulu, HI

Contract Number: N01-CB-53884
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To determine in postmenopausal women if there is an association of breast cancer with specific dietary factors and the use of menopausal estrogens.

Approach: A case-control approach is being used in which a questionnaire is employed to collect information on past history of drug usage (including menopausal estrogens) and usual weekly dietary intake. Breast cancer cases from three selected populations (ages 45-74) are identified: (1) the Caucasians in Hawaii who are at high risk for breast cancer; (2) the Japanese in Hawaii who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan, representing a low-risk population. For each case, a neighborhood and hospital control matched by race and age is interviewed. Attempts are made to verify the drug history with the subject's personal physicians, and a subsample of the study population receives a repeated dietary interview to assess its reliability.

Progress: As of August 5, 1977, interviews in Hawaii have been completed on 134 cases, 122 hospital controls, and 124 neighborhood controls. In Japan, 91 cases, 91 hospital controls, and 91 neighborhood controls have been interviewed, as of April 30, 1977.

Significance for Cancer Research: (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.; David Levin, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $85,000 Site Visit Date: 3/14/77

CONTRACT RESEARCH SUMMARY

Title: Benign and Non-invasive Breast Lesions in Groups at Different Breast Cancer Risk

Principal Investigator:	Dr. Sue Amelia Bartow
Name/Address	University of New Mexico
Performing Organization:	School of Medicine Albuquerque, NM

Contract Number: N01-CB-84231

Starting Date: Expiration Date:

Goal: To investigate whether population groups with low incidence of breast cancer also have a low prevalence at autopsy of benign breast lesions.

Approach: Combining the resources of the population-based New Mexico Tumor Registry, the State's Medical Investigator's Office and the Medical School Department of Pathology, we propose to examine and compare benign breast lesions in three ethnic groups in an autopsy series - Anglos, Spanish Americans, and American Indians. These three groups are all at differing risk for breast cancer, and the incidence rates and histopathologic distributions of breast cancers are already known from Tumor Registry data. The autopsy series to be examined will include about 500 women in three years, 230 Anglo, 140 Spanish American, and 130 American Indian. Breast lesions will be classified pathologically. Medical, reproductive, and related history of each case will be obtained from the family if at all possible.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.

Program: Breast Cancer Epidemiology

Technical Review Group: Breast Cancer Task Force Committee

FY 78 Funds: $77,000 Site Visit Date: 5/11/78

CONTRACT RESEARCH SUMMARY

Title: Relationship of Thyroid Function to Growth of Mammary Tumors

Principal Investigator Name/ Address: Performing Organization:	Dr. A. David Goodman Albany Medical College Albany, NY

Contract Number: N01-CB-74107
Starting Date: 8/15/77 Expiration Date: 8/14/79

Goal: To determine whether hypothyroidism is a significant risk factor for breast cancer.

Approach: DMBA will be administered intragastrically, intravenously, or directly applied to inguinal mammary glands in Sprague-Dawley rats, before, or shortly after induction of hypothyroidism by iodine deprivation or propylthiouracil administration, to study the effect of hypothyroidism on the initiation and growth of DMBA-induced mammary cancers. Estrogen-dependence and prolactin binding of the DMBA-induced tumors will be examined in hypothyroid and euthyroid animals. The effect of TSH on the DMBA-induced cancer also will be studied.

Progress: Moderate hypothyroidism, produced by placing proyplythiouracil (PTU), 1 mg%, in the drinking water, caused mild impairment of weight gain but had no effect on the growth of DMBA tumors. Severe hypothyroidism, produced by 4 mg% PTU, markedly impaired weight gain and totally prevented development of DMBA tumors. When rats receiving 4% mg PTU were also given T4 and T3, there was no hypothyroidism or reduction in weight gain, and the incidence of DMBA-induced tumors was the same as in control rats. The effect of mild hypothyroidsm, which does not affect weight gain, is currently being studied, as are the effects of alterations in thyroid function produced by variations in iodine intake.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $37,000 Site Visit Date: 4/12/77

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acids on the Biological Properties of Mammary Cancer

Principal Investigators: Drs. Janet S. Butel and Florence Farber
Name/ Address Baylor College of Medicine
Performing Organization: Houston, TX

Contract Number: N01-CB-53904
Starting Date: 4/1/75 Expiration Date: 3/31/79

Goal: To determine the relationship between transfer of nucleic acid preparations into cells and possible changes in their growth potential.

Approach: The approach has been four-fold: (1) To establish well-characterized mouse mammary tumor cell lines in culture; (2) To determine growth parameters of the tumor cells to monitor in vitro; (3) To characterize and optimize conditions for uptake of exogenous DNA by mammalian cells; and (4) To determine the effect of exogenous DNA on the growth properties of mammary tumor cells.

Progress: Cell lines have been established in tissue culture from mammary tumors which arose in BALB/ c mice either spontaneously or after treatment with a hormone or a chemical carcinogen. Several clonal lines have been derived from each parental line, the majority of which were transplantable in syngeneic weanling mice. There was no single growth property in vitro, which correlated with transplantability in vivo. The ability of the cells to multinucleate in the presence of cytochalasin B does appear to be an indicator of transplantability with the mammary tumor cell lines. Membrane alterations associated with transformation in other systems and transcription of MMTV sequences are also being investigated with the mammary tumor cell lines. The effect of normal cell DNA on selected growth properties of two clonal mammary tumor cell lines is currently under investigation.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V., Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $85,500 Site Visit Date: 8/6/74

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Neoplasia of the Mammary Gland

Principal Investigator:	Dr. Daniel Medina
Name/Address	Baylor College of Medicine
Performing Organization:	Houston, TX

Contract Number: N01-CB-43907
Starting Date: 6/10/74 Expiration Date: 6/9/78

Goal: Determine the relationship between stromal and normal mammary epithelial cells on growth and differentiation of preneoplastic and neoplastic epithelial cells from the mouse mammary gland.

Approach: Develop and use mammary cell culture systems in which normal and neoplastic epithelial cells can be co-cultured in presence of mesenchymal cells. Study the effect of stromal and normal mammary cells on the growth of the characterized cells in co-culture or in filter culture. Examine the interactions of normal and preneoplastic mammary epithelial cells in the progression of neoplasms.

Progress: During the past three years, a series of experiments have investigated the interaction of normal epithelial and stromal cells on the growth of preneoplastic and neoplastic mammary epithelial cells _in vivo_ and _in vitro._ _In vivo,_ several experiments have demonstrated that normal mammary epithelial cells can inhibit the neoplastic transformation in preneoplastic nodule lines, when a mixture of both cell types are injected into the mammary fat pads of syngeneic mice. In contrast, neither normal mammary epithelial cells nor normal fibroblasts inhibit the growth of tumor cells under the same conditions. The experiments also demonstrated that enzymatic dissociation of the mammary nodule cells but not normal cells, enhanced the rate of the neoplastic transformation in several cell lines.

In a series of _in vitro_ experiments, a variety of cell lines was established from mouse and rat mammary tumors. Four culture systems were examined to determine the best system to examine the effects of stromal cells on the growth of preneoplastic and neoplastic mammary cells. These include organ cultures, monolayer co-cultures, transfilter cultures, and the media exchange systems. The transfilter cultures appear promising, although several technical problems still need to be solved. The medial exchange experiments are establishing several parameters which will help us predict cell behavior in the modified Rose chambers. The more promising cell lines and clones derived from them are mammary tumors derived from GR mice and $(C57BLXDBAF)F_1$ mice. These tumors are hormone dependent _in vivo_, are transplantable in $(C57BLXDBAf)F_1$, grow out as epithelial cells _in vitro_, and so far are hormone responsive. The majority of the _in vitro_ work over the next 12 months will involve these cells.

Significance to Cancer Research: (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Richard A. Knazek, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/15/74

CONTRACT RESEARCH SUMMARY

Title: Effects of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Name/Address Performing Organization:	Dr. Jeffrey M. Rosen Baylor College of Medicine Houston TX

Contract Number: N01-CB 74189
Starting Date: 8/29/77 Expiration Date: 8/28/79

Goal: To study the influence of perinatal estrogen and progesterone administration on the development ane expression of differentiated function in the murine mammary gland.

Approach: Newborn BALB/c mice will be injected with estrogen or progesterone or with both hormones. The organ cultured mammary glands will be exposed to (a) insulin, hydrocortisone, and prolactin; (b) carcinogenic and non-carcinogenic analogues of DMBA; (c) thyroxine and growth hormone. Perinatal hormone treatment in virus-positive and -negative BALB/c strains of mice will be compared. In addition, the effect or pretreatment with estradiol and progesterone for either 6 or 9 days at 4 or 5 weeks of age, prior to organ culture will be compared. Morphological and biochemical analysis, including determinations of casein mRNA, and casein will be done.

Progress: Altered morphologic responses were observed after culture in tissue of BALB/c mice injected as newborns with estradiol or with estradiol and progesterone. Progesterone did not only affect morphologic responses. Both alevolar and lobular responses were enhanced as the duration of pretreatment was increased from 6 to 9 days. Minimal development occurred after culture with insulin alone. Culture with cortisol, prolactin, and insulin in combination resulted in maximal alevolar and lobular development. In agreement with these morphological data, perinatal estrogen administration resulted in an enhanced induction of casein mRNA in the presence of insulin, cortisol and prolactin, while perinatal progesterone treatment inhibited casein mRNA accumulation compared to untreated controls. A combination of insulin, hydrocortisone, and prolactin was also required for maximal induction of casein mRNA and the effects of perinatal treatment were increased when a pretreatment period of 9 days was employed prior to culture. These results suggest that perinatal estrogen, but not progesterone, may result in precoious development of the mammary gland, perhaps by a direct action at the tissue level.

Significance to Cancer Research: (NCP Objective 1 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $75,000 Site Visit Date: 3/1/77

CONTRACT RESEARCH SUMMARY

Title: Pathological History of Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigator: Drs. Susamu Ohno, James Kan
Name/Address: City of Hope National Medical Center
Performing Organization: Duarte, CA

Contract Number: N01-CB-33097
Starting Date: 6/27/73 Expiration Date: 6/26/78

Goal: To study the underlying mechanism of the inhibitory action of testosterone on mammary gland development by the use of the X-linked Tfm (testicular feminization) mutation of the mouse.

Approach: Since this is a summary of the last five years, this report deals only with findings of major significance, not limiting itself to mammary gland development. Products of two major regulatory genes that govern the mammalian sex determining mechanism have now been identified. The primary (gonadal) sex is determined by the expression or nonexpression of an evolutionary conserved plasma membrane antigen serologically detectable as H-Y antigen. Testis-organizing H-Y antigen is the first plasma membrane component to which a specific organogenesis-directing function has been assigned. In all mammals, testosterone synthesized by the H-Y antigen organized testis induces all the extra-gonadal masculine development, feminine development spontaneously occurring in the absence of testosterone. The androge responsiveness of every target cell type is governed by the X-linked Tfm locus which apparently specifies the one and only species of the nuclear-cytosol androgen-receptor protein.

Progress: 1) The identical mutation defect (80 to 90% reduction in the level) has been observed on the nuclear-cystosol androgen-receptor protein of every target cell type of affected XY (Tfm/Y) male pseudohermaphroditic mice. 2) Neonatal masculinization of hypothalmus and other components of the central nervous system, however, appears to be the function of in situ converted estradiol and its specific receptor, rather than androgen and its Tfm specified receptor. 3) Contrary to popular belief, the maturation of male germ cells does not appear to depend upon androgen. 4) As shown, the testis-organizing H-Y antigen, as well as an envelope protein, gp70, of oncogenic viruses use B_2-microglobulin-H-2 antigen complexes as their plasma membrane anchorage sites. The theory of competitive displacement as the oncogenic viral strategy has been proposed.

Significance to Cancer Research: (NCP Objective 1 Approach 4)

Project Officer: Chester V. Piczak, B.S., E Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/10/73

CONTRACT RESEARCH SUMMARY

Title: Change in Mammary Sensitivity to Carcinogens Related to Host Physiologic State

Principal Investigator: Name/Address Performing Organization:	Dr. Dilip K. Sinha Health Research, Inc. Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-74105
Starting Date: 8/29/77 Expiration Date: 8/28/79

Goal: To investigate the susceptibility of the mammary gland to neoplastic transformation during changing conditions of the host due to age and pregnancy.

Approach: Study the DNA synthesis of ductal and alveolar cells of prepubertal, pubertal, and postpubertal rats. Attempt to induce growth in mitotically static old mammary glands by transplantation. Determine the proliferative pattern of "old" and "young' mammary glands transplanted into the gland-free mammary fat pad. Attempt to induce tumors in old mammary glands transplanted into the gland-free fat pads of old rats.

Progress: The growth and development of mammary gland in prepubertal, pubertal, and postpubertal (aging) rats has been studied. 10-, 15-, 25-, 35-, 55-, 70-, 90-, 150- and 250-day-old female rats were used in this investigation. The developmental pattern is established by studying whole mount preparations of the abdomino-inguinal gland and by DNA synthetic index as indicated by ^{3}H-thymide uptake (labeling index). Mammary glands in 10-day-old rats start as single, branched duct with club-shaped end buds, which with age develop into multiple ductal system and ultimately almost fill up the fat pad. The proliferative index reaches its peak in 25 and 35 days; it then starts to decline. At 150 days and after, the glands are almost at stasis, considering proliferation. The second approach of this project was to investigate the proliferation and growth of pieces of mammary glands transplanted into isologous hosts. Small pieces of mammary tissue from 60-day-old rats were transplanted into the gland-free fat pad of 200-day-old rats and vice versa. Graft-bearing rats were killed at 60 days, 90 days, 120 days, and 150 days after transplantation. The growth pattern is observed by whole mount preparation and ^{3}H-thymide uptake (labeling indexes). Further investigations are necessary to obtain meaningful data.

Significance to Cancer Research: (NCP Objective 3 Approach 2,3)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $55,300 Site Visit Date: 3/22/77

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Dr. Richard C. Moon
Name/ Address IIT Research Institute
Performing Organization: Chicago, IL

Contract Number: N01-CB-74207
Starting Date: 8/29/77 Expiration Date: 8/28/79

Goal: To study the use of retinoids to prevent the development and/ or progression of preneoplastic lesions of the mammary gland.

Approach: Non-toxic doses of synthetic analogues of vitamin A (retinoid) will be administered to experimental animals in the diet to study: the effect on incidence of HAN and spontaneous mammary tumors in C3H/ He mice DMBA-induced hyperplasias and tumors in BALB/ c mice. The prevention of the progression of these ductal lesions and DMBA-induced mammary tumors are planned. Similar studies are planned using the DMBA and/ or MNU induced lesions in the Sprague-Dawley rat. The effect of retinoids on carcinogen uptake and binding and effect of time of initiation of retinoid treatment on DMBA or MNU induced carcinogenesis in the Sprague-Dawley rat will be examined. Studies will be designed to investigate the mechanisms by which retinoids are effective.

Progress: Studies have been initiated to determine the effect of retinoids (retinyl acetate, retinyl butyl ether, retinyl methyl ether, and 4-hydroxyphenyl retinamide) incorporated into the diet on the incidence of spontaneously occurring HAN and mammary tumors in C3H/ He mice and on DMBA-induced and MNU increase in DNA synthesis resulting from carcinogen treatment as evidenced by a decrease in incorporation of ^{3}H-thymidine into mammary parenchymal cell DNA. Furthermore, the effect was maintained over a 5-week observation period. Other studies indicate that retinyl acetate apparently prevents the progression of carcinogen-induced early neoplastic lesions and that a continuous daily intake of the retinoid must be maintained to sustain the inhibitory effect on progression.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $85,700 Site Visit Date: 4/21/77

CONTRACT RESEARCH SUMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Name/Address Performing Organization:	Dr. Klaus Kratochwil Institut für Molekularbiologie Salzburg, Asutria

Contract Number: N01-CB-33883
Starting Date: 6/29/73 Expiration Date: 3/28/79

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland. To analyze the androgen response with special emphasis on the role of tissue interaction.

Approach: Determine androgen-sensitive phase of mammary rudiment by exposing explanted mammary buds of various developmental stages to testosterone *in vitro*. Determine the target tissue for the hormone by experimental tissue combinations (gland epithelium and surrounding mesenchyme) taking advantage of the androgen-insensitive Tfm-mutant. Find out how the primary target tissue affects its partner (methods: experimental tissue combinations, electron microscopic and histochemical study of the response itself, autoradiography of suitable precursors - especially of tissue interface material). Determine whether the duration of the androgen-sensitive phase of the gland is specified by transient hormone responsiveness of the (primary) target tissue or by changing properties of its partner (hormone binding in relation to responsive phase, combination of tissues in different developmental stages). Compare androgen response in different mammalian species.

Progress: Established that testosterone response *in vitro* identical to the reactions *in vivo*. The temporary androgen-sensitive phase in mammary gland development was found to reflect intrinsic developmental changes in the gland itself. Established that the target tissue for testosterone is the mesenchyme, the necrosis of the gland epithelium - as seen in the electron microscopic study - must therefore be caused by testosterone-activated mesenchymal cells. These mesenchymal cells discriminate precisely between mammary and other epithelial cells. Although it was found that the response can be initiated only by mesenchymal cells immediately adjacent to the gland epithelium, and that breakdown of basal lamina precedes epithelial necrosis, the mechanism of the "mesenchymal attack" remains unknown. The response of the gland in mice and rats is similar; the rabbit gland appears to be unre--sponsive.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $72,000 Site Visit Date: 9/20/73

CONTRACT RESEARCH SUMMARY

Title: Influence of Mammary Carcinoma Cells on the Formation and Absorption of Collagen

Principal Investigator: Name/Address Performing Organization:	Dr. Gehard Wirl Institut für Molekularbiologie Salzburg, Austria

Contract Number: N01-CB-74109
Starting Date: 9/1/77 Expiration Date: 8/3/79

Goal: To isolate and characterize collaganese and inactive forms of collagenase present in mammary tumor tissue.

Approach: Studies are designed to: (1) extract collagenase directly from DMBA-induced tumors in the rat and spontaneous tumors in C3H mice as well as from tumor tissue cultured media; (2) purify the enzyme and examine its purity using disc gel electrophoresis and immodiffusion; (3) detect the activity of the enzyme; (4) determine the distribution of the enzyme in the tumor; (5) find any inactive form of the enzyme as a proenzyme; (6) determine the production of collagenase by epithelial cells or stromal cells. Studies of human tissues depends on results obtained from the mouse and rat.

Progress: Simple homogenization of DMBA-induced tumors of the rat in either 0.4 M sucrose or 1.0 M NaCl, buffered in Tris-HCL, pH 7.6, is sufficient to bring one part of total tissue collagenase into solution. Additional enzyme could be extracted from the 6000 x g sediment of the tumor homogenate by the use of 5 M urea. This enzyme has been partially purified by gel filtration and affinity chromatography showing a molecular weight of approximately 60,000. No detectable change in activity occurred after treatment of the various extracts with trypsin or 3 M NaSCN, suggesting the absence of latent collagenase.

Preliminary observations indicate that both epithelial and connective tissue cells derived from DMBA-induced tumors release small amounts of collagenase into the serum-free medium. Parallel attempts to detect collagenase in the medium of organ cultures are still unsuccessful and studies are in progress to the culture conditions. However, tumor explants exhibit collagenolytic activity when grown on collagen gel.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $45,000

CONTRACT RESEARCH SUMMARY

Title: Animal and Human Mammary Tumors and Cell Culture Bank Facility

Principal Investigator:	Dr. Arthur E. Bogden
Name/Address	Mason Research Institute
Performing Organization:	Worcester, MA

Contract Number: N01-CB-74175
Starting Date: 6/28/63 Expiration Date: 6/27/79

Goal: To function as a service facility for the cryopreservation, storage, and distribution of biologically characterized and monitored (a) animal and human mammary and endocrine related tumors transplantable in vivo and (b) cell lines of human and animal breast tumor origin established in vitro culture, for use by BCTF and other selected investigators.

Approach: Cryopreservation of mammary and endocrine related tumors of animal and human origin, as well as human breast tumor cell lines: (a) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (b) biological characterization of transplantable tissues, both pre- and post-cryopreservation, by determination of growth curves, specific effects on host organs, host (syngeneic and xenogeneic) survival, serum hormone levels, histology, karyology, response to ablative procedures; (c) cell lines tested for viability, plating efficiency, freedom from contamination, and tumorigenicity by nude mouse implantation; (d) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to well recognized, proven procedures.

Progress: During the past contract period, 136 tumors have been shipped to 74 investigators; 43% were of mammary and 18% of anterior pituitary origin. There are 188 different tumors or sublines stored in 3,759 ampules in liquid nitrogen. Sixty-seven new tumors and 36 tumor lines have been maintained in serial transplantation for characterization and/or replenishment of Tumor Bank inventory. There are now cryopreserved, characterized, and free of mycoplasma, 13 human mammary cell lines as well as the WI-38 and the 13762 rat mammary ascites tumor line. Forty-nine shipments that include viable cultures, frozen ampules and/or gram quantities of packed cells, have been made to 25 different investigators. Our isolation facility for housing and breeding athymic (nude) mice has been expanded. Five human breast tumors established in serial transplantation as xenografts have been cryopreserved. These are being characterized and are avaialble for distribution. Also available are rat and human α-lactalbumin and specific antisera prepared and submitted by Dr. Kurt Ebner.

Significance to Cancer Research: (NCP Objective 3,4,5 Approach)

Project Officers: Chester V. Piczak, B.S.; D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $170,200 Site Visit Date: 3/4/71

CONTRACT RESEARCH SUMMARY.

Title: Identification of Mammary Tissues

Principal Investigator: Name/Address Performing Organization:	Dr. Melvyn S. Soloff Medical College of Ohio Toledo, OH

Contract Number: N01-CB-63983
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To isolate, identify, characterize, and quantitate myoepithelial cells in rat mammary tissue and tumors of rat and human origin.

Approach: Separate myoepithelial cells by centrifugation in albumin gradients, using oxytocin binding, and scanning/transmission electron microscopy to identify the cells. Examine the potential of using alkaline phosphatase and ATPase as cell markers. Prepare specific antiserum and identify, quantitate, and characterize myoepithelial cells in mammary glands from virgin, pregnant, and postpartum rats, and in rat mammary tumors induced with DMBA. Parallel investigations will be carried out with human tumors obtained from surgery.

Progress: Oxytocin binding by particulate fractions from rat mammary glands was found to increase progressively through pregnancy and plateau during lactation. Myoepithelial cells isolated from lactating glands could be discriminated from other mammary cell types by $[^3H]$ oxytocin binding, alkaline phosphatase cytochemistry, T.E.M., and S.E.M. Isolated myoepithelial cells appear viable by the ability to contract in response to the addition of oxytocin.

Myoepithelial cells and secretory cells have been purified and are being used to immunize rabbits. Sera obtained from initial bleedings are being tested by immunofluorescence and peroxidase-antiperoxidase techniques.

Preliminary evidence from studies on $[^3H]$ oxytocin binding to particulate fractions from human mammary/tumors indicates that binding occurs in fibrocystic disease with significant sclerosing adenosis, but not in adenocarcinoma.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $88,300 Site Visit Date: 2/13/76

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator:	Dr. Clifford W. Welsch
Name/Address	Michigan State University
Performing Organization:	East Lansing, MI

Contract Number: N01-CB-74090
Starting Date: 8/29/77 Expiration Date: 8/28/78

Goal: To prevent the early development of preneoplastic mammary gland lesions and progression of these preneoplastic lesions to neoplastic lesions by chronic suppression of pituitary prolactin secretion.

Approach: A prolactin-suppressing drug, 2-bromo-α-ergocryptine (CB-154), will be administered prior to and after the spontaneous development of, or the induction of, mammary gland dysplasias. The following mammary gland dysplasias will be examined: A. Mice: 1) spontaneously occurring alveolar lesions (HAN) - DBA/2; 2) pregnancy-induced alveolar lesions (HAN) - A/J; 3) DMBA-induced lesions (DH) - BALB/c; 4) pregnancy-induced ductal lesions (plaques) - GR/A. B. Rats: 1) DMBA-induced ductal lesions - Lewis and Sprague-Dawley; 2) X-irradiation-induced ductal lesions - Lewis and Sprague-Dawley; 3) DMBA-induced alveolar lesions - Lewis and Sprague-Dawley; 4) X-irradiation-induced alveolar lesions - Lewis and Sprague-Dawley; 5) pregnancy-induced alveolar lesions - Sprague-Dawley.

Progress: New Contract: All of the rodent species have been received and are now on experimentation. Since nearly all of these studies are long-term, few experimental results at this point in time have been obtained. The only exception to this is the studies using carcinogen-treated Sprague-Dawley rats. Chronic prolactin suppression at the time of carcinogen treatment reduced mammary carcinoma incidence by 40%.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $83,700 Site Visit Date: 4/21/77

CONTRACT RESEARCH SUMMARY

Title: Metabolism of Polycyclic Aromatic Hydrocarbons in the Induction of Mammary Tumor

Principal Investigator:	Dr. A. Monaem El-hawari
Name/Address	Midwest Research Institute
Performing Organization:	Kansas City, MO

Contract Number: N01-CB-84227
Starting Date: Expiration Date:

Goal: To determine whether the disposition, metabolism, and binding are responsible for differences in mammary tumor incidence caused by polycyclic aromatic hydrocarbons.

Approach: Experiments will be done on the following eight groups of rats: Mature virgin females from Sprague-Dawley, Long Evans, and Lewis strains; mature male rats from Sprague-Dawley strain; young, old, pregnant, and lactating rats from Sprague-Dawley strain. Tumors will be induced with either 3-MC(66 mg/100g) or DMBA (13.3 mg/100g). Control animals will receive sesame oil (1 ml/100g). Disposition and metabolism studies will be done both in vivo and in vitro. Covalent binding to macromolecules and various enzyme activities will be determined.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 1 Approach 3)

Program Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 4/24/78

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of the Mammary Cell Surface

Principal Investigator: Dr. Eugene A. Davidson
Name/Address The Milton S. Hershey Medical Center
Performing Organization: The Pennsylvania State University
Hershey, PA

Contract Number: N01-CB-63984
Starting Date: 6/20/76 Expiration Date: 6/19/79

Goal: To isolate and characterize the cell surface glycoproteins and other complex carbohydrates produced by human mammary carcinoma cells.

Approach: Apply techniques for surface labeling of the protein and carbohydrate moieties of the glycoproteins on the cell plasma membrane. Assess the labeled macromolecules in terms of molecular size and localization within the membrane. Apply the best method for isolation and purification of plasma membranes. Characterize the purified plasma membrane for their chemical architecture, biological, immunological properties, and enzyme markers. Human mammary cell lines obtained from the BCTF Cell Bank will be used for the studies.

Progress: Studies are continuing with MDA-MB-231, MCF-7, and HBL-100 cell lines. The former two (mammary carcinoma) lines produce significantly more mucin-type sialoglycoproteins than do the normal HBL-100 cells. These proteins have been isolated by affinity chromatography on wheat germ agglutinin-Sepharose columns. After treatement with alkaline borohydride, the eliminated oligosaccharide products have been separated and partially characterized. The major saccharide studied is larger in size than a marker tetrasaccharide alcohol prepared from fetuin and contains galactose, sialic acid, and glucosamine in addition to galactosaminitol. The glycosaminoglycan products of all cell lines have been characterized. The MDA-MB-231 cells have been inoculated into athymic mice, and solid tumors developed with characteristic mammary carcinoma histopathology. The HBL-100 cells do not grow under identical conditions; the MCF-7 line produces nodules that develop rather slowly into tumors. Studies of the glycoproteins present in the solid tumors as well as those which may be tumor derived but appear in host circulation are continuing.

Plasma membranes have been isolated from all lines, and partial characterization has been accomplished. Antibodies have been prepared against glutaraldehyde fixed MDA-MB-231 cells. These have been fluorescein labeled and shown to react at the cell surface. Common determinants are present for all three lines and specificity studies are underway.

Significance to Cancer Research: (NCP Objective 3 Approach 5)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $123,000 Site Visit Date: 2/17/76

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator: Dr. Burton Goldberg
Name/Address New York University Medical Center
Performing Organization: New York, NY

Contract Number:
Starting Date: Expiration Date:

Goal: To produce specific antibodies to the carboxyterminal non-helical sequences of human type I procollagen in sufficient quantities for storage and distribution to other investigators.

Approach: Culture human skin fibroblasts (CRL1121) in roller bottles and isolate the procollagen in the culture medium then subject it to specific bacterial collagenase digestion. Purify the carboxyterminal fragment by ion exchange chromatography then produce antibodies in appropriate animal species (rabbit, goat, sheep). Purifiy the antisera by absorption techniques and/or affinity chromatography and confirm their specificity by Ouchterlony technique, immunoelectrophoresis, passive hemaglutination and radioimmunoassay.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 4/27/78

CONTRACT RESEARCH SUMMARY

Title: Development of an Assay for Genetic Damage to Mammary Gland Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Ronald W. Hart Ohio State University Hospitals Columbus, OH

Contract Number: N01-CB-84226
Starting Date: Expiration Date:

Goal: To develop an inexpensive, rapid and quantitative assay for detecting genetic damage and its repair in mammary cells in vivo following exposure to carcinogenic agents.

Approach: Measure DNA damage directly by an assay using S_1 endonuclease. Determine changes in the size of non-radiolabelled DNA resulting from endonuclease treatment using alkaline or neutral sucrose gradient sedimentation. After sedimentation, the non-radiolabelled DNA will be quantified by a spectrofluorometric technique. Quantify the mammary epithelial cell DNA damage by double label autoradiography. DMBA sensitive and resistant rat strains and non-carcinogenic and less carcinogenic analogs 2-FL-DMBA and 5FL-DMBA will be used for these studies.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 3 Appraoch 2)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 5/4/78

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Mammary Tissue

Principal Investigator: Dr. Edwin V. Gaffney
Name/Address Pennsylvania State University
Performing Organization: University Park, PA

Contract Number: N01-CB-33898
Starting Date: 6/29/73 Expiration Date: 8/31/78

Goal: Establish monolayer cultures of human mammary epithelial cells derived from normal, benign, and malignant human breast tissues. Examine the influence of different hormones and serum factors on cell growth and functional capacity.

Approach: The effect of various cell factors on growth is measured by using colony formation by cell clusters, cell number, DNA and protein synthesis. Stuuctural changes are followed by light and electron microscopy.

Progress: Cultures composed solely of epithelial cells are established from milk and tissue samples. An epithelial cell type, E, is identified in cultures established from all sources. A second cell type E', is found only in cultures from ductal carcinomas. Hydrocortisone, which increases cell growth, has no mitogenic response with E' cells. Prolactin and insulin both stimulate ^{3}H-thymidine incorporation into cells from normal sources. In addition, prolactin stimulates phosphoprotein synthesis and induces ultrastructural changes indicative of differentiation.

Clusters of cells from malignant tumors attach to plastic surfaces with a low frequency. The majority of colonies are composed of E' cells, although E cell and fibroblast colonies are observed when collagenase is used. A comparison of various sera has shown that colony formation was maximum in the presence of fetal calf serum. Human serum alone was 70% to 100% less effective in promoting E' colonies. The most significant finding was that human serum from normal donors inhibited E' colony development in the presence of fcs. Although human serum was less effective than fcs in promoting colony formation by clusters of E cells, an inhibition was not observed.

Inhibition cannot be attributed to either seurm source, hormones, complement related antibodies, or lipoproteins. Sera from patients with ductal carcinomas do not inhibit colony formation by cells from mammary tumors. Inhibition is diminished by incubation of serum with malignant cells prior to use in colony formation studies. Certain fractions of human serum contain proteins which complement the effects of fetal calf serum.

Efforts are continuing to delineate similarities among cells from mammary tissues, to explore the relationship between certain hormones and sera, and to test for growth enhancement or inhibition by serum factors.

Significance to Cancer Research: (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Katherine Stanford, Ph.D
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/27/73

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis

Principal Investigator: Name/Address Performing Organization:	Dr. Wesley C. Hymer Pennsylvania State University University Park, PA

Contract Number: N01-CB-23863
Starting Date: 6/28/72 Expiration Date: 9/28/78

Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach: The following material will be used: (1) human pituitaries from surgical material, and (2) rat pituitaries from animals bearing either 13762 mammary adenocarcinomas (ascites and solid form) or R-3230 tumors. Experiments include: (1) cell dissociation, (2) cell separation by velocity sedimentation at 1G (NIH model chamber), (3) cell cultures, and (4) radioimmunoassay of pituitary hormones.

Progress: I. Culture of prolactin cells. Pitutitary cells from ovariectomized Sprague-Dawley rats were cultured for 9 days in 20 different commercially available synthetic media containing either 5% horse or calf serum. Widely varying patterns of prolactin secretion were noted, ranging from 100% (optimal medium) to 5% (poorest medium). In optimal medium the cells renewed their hormone content 24.5 ± 2.9 times during the 9 day culture period. We succeeded in maintaining actively secreting mammotrophs in serum-free medium for 1 week by the addition of physiological concentrations of T_4 and E_2 to the medium. These results suggest that hormones in serum probably plan an important role in maintaining mammotroph activity *in vitro*. Parallel studies of human pituitary mammotrophs isolated from glands of breast cancer patients are ongoing. In general, the results appear similar to those obtained with the rat cell models. II. Ascites fluid (AF) and prolactin secretion *in vitro*. Based on our previous observation that prolactin secretion from pituitary cells in culture was enhanced by addition of normal mammary cells to the dish, we tested the effects of cell-free AF obtained from rats bearing the 13762 mammary ascites adenocarcinoma. A prolactin stimulating factor was found in AF that was active on pituitary cells maintained either in the absence or presence of serum. Activity was related to dose of AF used, and was destroyed by boiling or treatment with trypsin. The responsiveness of the pituitary cell to the active agent(s) appears partially dependant upon the physiological state of the animal. These results are consistent with the hypothesis that the mammary gland may participate in the regulation of anterior pituitary gland function.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 10/19/76

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator:	Dr. W. B. Dandliker, and R. J. Brawn
Name/Address	Scripps Clinic and Research Foundation
Performing Organization:	La, Jolla, CA

Contract Number: N01-CB-43905
Starting Date: 6/1/74 Expiration Date: 2/28/79

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones. Investigate possible competitive, cooperative, or sequential effects in the binding of hormones to their specific cellular receptors of mammary tissue. Based upon these findings, develop simple fluorescence receptor assays useful in the clinical laboratory.

Approach: Fluorescent tagging of hormones, especially the steroid hormones, purification of the fluorescent derivatives by chromatography, and the testing of their binding activity by the dextran-coated charcoal method, by fluorescence polarization and by fluorescence microscopy. Specificity in each case is defined by the ability of the fluorescent labeled hormone to compete with estradiol in binding to receptor preparations, cells, or tissue sections from mammary tumors as compared to normal controls from hormone responsive or nonresponsive tissues.

Progress: Several fluorescent estrogen derivatives have been made, and studies on these are directed toward delineating the important structure-function parameters which determine the degree of binding activity. The most crucial structural requirement of high binding affinity seems to be the preservation of the integrity of the A and B rings and of the 3-OH group. Quantitative data on receptor content for normal tissues including uterus, liver, bladder, kidney, spleen, pregnant mammary tissue, and for tumor tissue including the R-3230 AC 13762 ascites, DMBA #8, DMBA #14, and MTW9 have been obtained by the DCC method. Values for estrogen receptor content for normal immature uterus are much higher than for any other tissue tested. Fluorescence polarization equilibrium data expressed as Scatchard plots have now been found to be amenable to a new simple mathematical analysis, which quickly gives the binding affinity and number of specifically binding sites. These measurements along with those of dilution jump furnish the most practical means for applying fluorescence polarization to hormone receptor studies. An extensive account of this entire methodology and for the necessary instrumentation will appear in a forthcoming volume of Methods in Enzymology. Fluorescence microscopy using several fluorescent estrogens show that there are important factors now under investigation which determine the type of staining pattern obtained when whole cells or tissue sections are concerned. These factors, involving the molecular structure of the fluorescent estrogen, probably include molecular net charge, hydophobicity and molecular size.

Significance for Cancer Research: (NCP Objective 3 Approach 3)
Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $67,000 Site Visit Date: 3/13/74

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acids on Biological Properties of Mammary Cancer

Principal Investigator: Name/Address Performing Organization	Dr. Aaron Bendich Sloan-Kettering Institute for Cancer Research New York, NY

Contract Number: N01-CB-43904
Starting Date: 6/28/74 Expiration Date: 3/27/78

Goal: To develop lines of normal and tumor cells appropriate for study of oncogenesis, to examine breast cyst fluids and their exfoliated cells for biochemical evidence of retravirus DNA polymerases, and to test sera from breast cancer patients for antibodies reactive with these polymerases.

Approach: To maintain or propagate epithelioid cells isolated from cyst fluids and from normal and tumor tissues. Solid tissues will be diasggregated enzymatically, culture media added, and various growth-enhancing additives tested. Cyst fluids will be separated from the various cells they contain, and these will be partitioned into cells that attach to culture vessel surfaces under tissue culturing conditions and those that don't. The identification and the ability of the cells to elaborate terminal transferase and endogenous RNA-instructed DNA polymerase activity will be determined with and without treatment with carcinogens and promoters. The possible presence of these enzymes in the cyst fluids will also be analyzed. Sera from normals and from breast cancer patients will be tested for their ability to inhibit these and retraviral enzymes.

Progress: Five breast cyst fluids and the tissue culture media derived from cultured cells isolated from the fluids were examined for biochemical evidence of retraviruses or their components. In all five cyst fluids, a high-speed particulate fraction with sucrose density ~1.16 g/ml was found that possessed endogeneous DNA polymerase activity; terminal deoxynucleotidyl transferase activity was also found. In one of the cultures, a particulate DNA-synthesizing fraction (sucrose $\rho = 1.17$) was identified in the medium only after treatment of the cells with the carcinogen MNNG. In cell cultures from two other cyst fluids, similar particulate DNA-synthesizing complexes were produced that did not require MNNG treatment. IgG from sera of two breast cancer patients inhibited the DNA polymerase activity in a breast fluid.

Human sperm nuclei and seminal fluid were found to contain an endogeneous DNA polymerase activity. Antibodies (IgG) against human sperm nuclei inhibited not only the sperm and seminal fluid DNA polymerase activity, but that of the retraviruses BEV and RD-114 as well; that of SSV, R-MuLV, FeLV, and AMV were not inhibited. Possible relationships among the DNA polymerases of breast cyst fluid, semen, and retraviruses are under study to determine the significance of these enzymes in normal neoplastic processes.

Significance to Cancer Research: (NCP Objective 2, 2 Approach 3, 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 2/22/74

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Growth of Neoplasms of the Mammary Gland

Principal Investigator: Dr. Merton R. Bernfield
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: N01-CB-53903
Starting Date: 4/15/75 Expiration Date: 4/14/79

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG), and the stroma in postnatal mammary development and neoplasia.

Approach: Use mammary glands undergoing postnatal morphogenesis and mammary epithelial cell cultures to 1) establish the presence of basal laminar GAG; 2) elucidate its distribution, organization, and composition; 3) analyze its metabolism; and 4) relate these findings to epithelial morphogenesis and neoplastic invasion.

Progress: Established by histochemistry, autoradiography, and biochemical characterization that the mouse mammary epithelial basal lamina contains GAG. Ultrastructurally, with special staining for GAG, the lamina is composed of units in regular periodic arrays attached to the basal plasmalemma. Incorporation studies at several ages suggest that laminar GAG turns over most rapidly at sites of greatest morphologic change. Thus, the lamina appears to be an organized GAG-rich extracellular scaffolding, whose metabolism reflects epithelial development.

Developed an in vitro system for studying the biosynthesis and metabolism of the mammary epithelial basal lamina and its GAG. Secondary cultures of mouse (Namru) mammary epithelial cells are cultured in insulin-containing medium and analyzed for the incorporation of $^{35}SO_4$ into GAG associated with cells and in the medium. Cells maintained in both plastic and type I collagen gel substrata incorporate $^{35}SO_4$ into GAG which is predominantly in proteoglycan of $\geq 10^6$ MW. During 4 hours labeling, no difference is seen between the substrata in the rate of total ^{35}S-GAG accumulation. After 48 hours labeling, however, total accumulation in collagen gel cultures is substantially greater than in plastic cultures, exclusively due to a 2-3 fold greater accumulation of ^{35}S-GAG associated with the cells. In contrast to gel cultures, the cell-associated ^{35}S-GAG in plastic cultures reaches a steady state level of incorporation after 24 hours labeling and is lost at a 6-fold faster first-order rate during a chase. Thus, the collagen gel causes the accumulation of cell-associated GAG by retarding its degradation. Autoradiography and ultrastructure show that the cell-associated GAG accumulating in collagen gel cultures is in a $^{35}SO_4$-labeled, highly ordered, continuous basal lamina.

Present work is directed toward using this system to elucidate the mechanisms which underlie and control laminar GAG assembly and degradation.

Significance to Cancer Research: (NCP Objective 4 Approach 3)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $95,000 Site Visit Date: 9/5/74

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigator: Name/Address Performing Organization:	Dr. Zoltan Lucas Stanford University Stanford, CA
Contract Number: N01-CB-33905 Starting Date: 5/24/73	 Expiration Date: 5/23/78

Goal: To study natural mechanisms imparing cellular and humoral immunity to syngeneic tumors.

Approach: Purify and characterize the factors, in serum of tumor-bearing animals, which block cellular and humoral immunity to the sygeneic tumors.

Progress: Tumor antigen(s). The previous summary described the assay of tumor antigen in supernatants of monolayer cultures of 13762A mammary adenocarcinoma (MTM) by immunoprecipitation of antigen labeled with ^{3}H-fucose. Difficulty in duplicating some of those results has occurred since the MTM cells current to those experiments were accidentally lost. Antigen(s) in supernatants of MTM cells of earlier generation times can be detected if culture media contain protease-inhibitors such as Σ-amino caproic acid. These antigens differ from the previous antigens in incorporating less ^{3}H-fucose and in having a larger amount precipitated by non-immune Fischer sera. Electrophoresis of immune precipitates of non-ionic detergent extracts of tumor cells labeled with ^{3}H-leucine on SDS-polyacrylamide (PAGE) shows three polypeptide chains of approximate molecular weights 28, 22, and 15 x 10^3 daltons. Although normal sera precipitate the same polypeptides as serum 7 days after tumor inoculation, the relative proportions of the three polypeptides differ markedly. These results are too preliminary for interpretation. Tumor antigen is also detected by inhibition of lymphocyte-mediated cytotoxicity (CMC). Currently, we are studying whether the antigen precipitated by serum is the same as that inhibiting CMC. Anti-tumor antibody. Cytotoxicity assays using complement show that non-immune Fischer sera contain IgG lysing MTM cells. Contrary to expectations, the amount of cytotoxin decreases with i.p. inoculation of tumor. Factor blocking CMC. Fractionation of immune sera on Sephacryl on 8M urea does not dissociate the IgG blocking factor, consistent with its being an anti-receptor antibody. In summary: Problems with isolation of and assay for tumor antigen have delayed characterization of factors in sera of tumor-bearing rats blocking CMC and, possibly, also "natural" antibodies.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/12/73

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Culture of Normal Mammary
Epithelial Cells

Principal Investigator:	Dr. Dayton S. Misfeldt
Name/Address:	Stanford University
Performing Organization:	Stanford, CA

Contract Number: N01-CB-74094
Starting Date: 7/1/77 Expiration Date: 6/30/79

Goal: To isolate and maintain growth of pure normal mammary epithelial cells in culture.

Approach: Mammary glands from hormonally stimulated virgin mice and human breast tissue from mammoplasty surgery will be dissociated to yield highest seeding efficiency. To determine optimal growth and passage conditions, the following factors will be tested: (a) commercial media; (b) types and centrations of sera; (c) pH and pO_2; (d) culture dish substrata. Fibroblast growth will be controlled by use of selective media and enzymatic treatment. Optimal methods of monolayer release for passage will use enzymatic, chelation, and mechanical methods.

Progress: Using 5-6 week estrogen-deoxycorticosterone (E+DCA) stimulated 4 month old virgin Balb/c female mice, the method of mammary gland dissociation that gave the optimal yield of viable cells and the highest seeding efficiency in primary culture was determined. Twelve commercially available nutrient media and 5 types of sera (horse, pig, human, fetal calf, and bobby calf) over a concentration range of 5-20% were tested on primary cultures. High glucose Dubecco's Modified Eagle Medium (HGDMEM) containing 15% fetal calf, bobby calf, and horse serum gave the highest seeding efficiency at standardized plating densities. Media effects on growth to confluence were not as stringent since several media were equally effective.

Normal human breast tissue from 6 donors was dissociated in a variety of ways to determine the method which gave the highest yield of epithelial cells surviving in primary culture. These cells attached and grew optimally, in HGDMEM containing 15% fetal calf serum, whereas several lots of human serum proved less effective. In 2 separate trials, fibroblast overgrowth of the epithelial cells was arrested by maintenance in D-valine DMEM containing 10% fetal calf serum.

Significance to Cancer Research: (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $66,500

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator:	Dr. Frank E. Stockdale
Name/Address	Stanford University
Performing Organization:	Stanford, CA

Contract Number: N01-CB-23875
Starting Date: 6/1/72 Expiration Date: 5/31/78

Goal: To determine the role of serum factors and hormones in the initiation and control of DNA synthesis in mammary gland epithelium and mammary neoplasms of mice.

Approach: Studies include (1) the isolation, characterization, and source of factors in serum responsible for mammary epithelial growth; and (2) comparison of the proliferative response of normal and malignant mouse mammary gland epithelium *in vitro* or *in vivo* to mammary serum factor (MSF) and hormones.

Progress: A factor(s) was found in serum which initiates DNA synthesis in normal mouse mammary gland and in mouse mammary tumor epithelium. This factor--mammary serum factor (MSF)--is relatively heat stable and has been partially purified by ionic exchange chromatography, gel filtration, and isoelectric focusing. Partially purified MSF has a molecular weight of approximately 10,400 and an isoelectric point of 5.5 to 6.0. MSF can be isolated from a variety of mouse or rat sera. Its activity decreases at least 50% in the sera of hypophysectomized rats, and the sera activity is not restored by treating the hypophysectomized rats with estrogen, prolactin or growth hormone. We have shown either by radioimmunoassay, bioassay, competitive binding assays, or immunodiffusion that mammary serum factor is different from NGF, EGF, MSA, FGF, somatomedin A and C and insulin.

Present work includes: (1) Studies on further characterization of mammary serum factor. (2) Studies on the effects of injection of partially purified MSF on mammary gland and tumor growth. (3) Studies on the hypothesis that in terms of growth, the mammary gland is composed of two or more *operationally* different populations. (4) Studies on growth-promoting substances released from normal and malignant mammary epithelium.

Significance to Cancer Research: (NCP Objective 5 Approach 6)

Project Officer:. D. Jane Taylor, Ph.D., William Kidwell, Ph.D
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 4/10/72

CANCER RESEARCH SUMMARY

Title: Morphological Properties of Normal and Abnormal Human and Rodent Mammary Tissue

Principal Investigator: Name/Address Performing Organization:	Dr. Elinor Spring-Mills State University of New York Syracuse, NY

Contract Number: N01-CB-84239
Starting Date: Expiration Date:

Goal: To evaluate structural differences among normal dysplastic and cancerous mammary glands.

Approach: To describe, quantitate, and compare certain morphological and functional characteristics of normal, hyperplastic, and carcinomatous human and mouse mammary glands *in vivo* and *in vitro*. Human tissues obtained at biopsy, mastectomy, and reduction mammoplasty will be prepared for light microscopy (LM), transmission electron microscopy (TEM), scan electron microscopy (SEM), and quantitative electron microscopy (QEM) or morphometry. Animal tissue from non-pregnant (pubertal and adult), pregnant, and multiparous C3H mice will be studied.

Progress: New Contract

Significance for Cancer Research: (NCP Objective 4 Approach 2)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $70,000 Site Visit Date: 5/9/78

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Dr. Beppino C. Giovanella
Name/Address: The Stehlin Foundation
Performing Organization: Houston TX

Contract Number: N01-CB-33856
Starting Date: 2/1/73 Expiration Date: 1/31/79

Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential, hormone responsiveness, and their response to chemotherapeutic agents.

Approach: A colony of thymusless-nude Swiss mice has been developed, and the good health of the animals permits their use in long-term experiments. Each tumor sample is transplanted into at least 2 adult females and 2 adult males treated with antilymphocyte serum - AL(B)S-subcutaneously. If enough material is available, the tumor is transplanted subcutaneously and intracerebrally in adult untreated nude mice and subcutaneously and intraperitoneally in untreated newborn nude mice with and without a source of angiogenetic factor(s). If the tumor grows, it is re-transplanted for at least 3 generations. The transplanted tumors are examined histologically and characterized by a pathologist.

Progress: One hundred sixty-seven primary and metastatic human breast carcinomas and 37 cell lines from human breast cancers have breen transplanted in 2,734 nude-thymusless mice. Of 57 breast cancers transplanted directly from the patient into the untreated nudes, 28 grew slowly and only 1 or 1.8% could be transplanted serially. Until last year, of 54 tumors transplanted into AL(B)S treated mice, 30 grew and and 7% or 13% could be transplanted serially. During the past year, using improved preparations of AL(B)S, 10 of 21 breast carcinomas grew, and 6% or 29% could be transplanted serially. The intracerebral route of inoculation is actively investigated, and the early results are very encouraging. Heterotransplantation of breast carcinomas in untreated newborns and under the kidney capsule of adults has been less successful. Nineteen of 37 cell lines derived from human breast carcinomas grew after subcutaneous injection in untreated adult nude mice, and two of them produced metastases in the lymph nodes and in the lungs. Five serially transplanted breast carcinomas have been tested for their sensitivity to various anti-cancer drugs in 600 nudes. Each tumor exhibited an individual pattern of response to chemotherapy. Two tumor cell lines grew faster in males and two in females.

Significance to Cancer Research: (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force
FY 78 Funds: $149,700 Site Visit Date: 10/27/72

CONTRACT RESEARCH SUMMARY

Title: Influence of Carcinogens of Viral Gene Expression Leading to Mammary Cancer

Principal Investigator:	Dr. Asher Frensdorff
Name/Address:	Tel Aviv University
Performing Organization:	Tel Aviv, Israel

Contract Number: N01-CB-74118
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To study logitudinally the influence of chemical carcinogens alone and in combination with prolonged hormonal stimulation on murine mammary tumor virus-gene expression.

Approach: MuMTV gene expression will be studied by measuring three independent parameters: 1) levels of MuMTV-RNA in mammary gland cells at varying intervals after onset of oncogenic stimuli (post-transcriptional level); 2) levels of MuMTV-antigens expressed on mammary gland tissue and in host serum, milk, or as antigen-antibody complex deposits in the kidneys (postranslational level); 3) autogenous humoral immune response to MuMTV and to MuMTV polypeptides and other antigens. Oncogenic stimuli will be DMBA, NMU, radiation and prolonged hormonal stimulation. BALB/c mice will be used for these studies.

Progress: New Contract. This recently initiated project, is a longitudinal study of individual MuMTV-negative BALB/c mice in which mammary tumors (MTs) are induced by the oncogenic stimuli listed above, or by neonatal infection with endogenous or exogenous MuMTV. Induction protocols have been selected, which cause tumors in only part of the treated mice, to enable comparison of MuMTV expression in mice which ultimately develop MTs, with that of mice which do not. Only newborn mice of substrains BALB/cTau and BALB/cTau/fC3HHeN, which have been established by us recently especially for this study and which are bred in our own facility, are included in the study. Each of the 12 planned experimental groups consists of 100 female mice; the establishment of some of the groups consists of 100 female mice; the establishment of some of the groups has already been completed. Serum is obtained periodically from all mice and stored individually. No antibody to exogenous (RIII or C3H) MuMTV has been detected by RIA in samples from young mice tested so far. Simultaneously, MTs from MuMTV-O positive BALB/c mice have been obtained and are propagated *in vivo* and *in vitro*. Attempts to isolate MuMTV-O from these tumors are underway.

Significance to Cancer Research: (NCP Objective 1,2 Approach 1,3)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $112,755

CONTRACT RESEARCH SUMMARY

Title: Detection and Quantitation of Transformed Mammary Epithelial Cells *In Vitro*

Principal Investigator:	Dr. William Niedermeir
Name/Address	University of Alabama
Performing Organization:	Birmingham, AL

Contract Number: N01-CB-74092
Starting Date: 8/29/77 Expiration Date: 8/28/79

Goal: To identify and isolate mammary tumor related cell-surface antigens and use these antigens in biochemical assays to discriminate between normal and neoplastic human mammary epithelium.

Approach: Two approaches are being taken in an effort to identify tumor antigens present on adenocarcinomas of the breast. 1) Solubilization of tumor antigens is achieved by trypsinization, KCl extraction, or sonication. The solubilized glycoproteins are isolated and purified by ion exchange and affinity chromotography. Radioimmunoassay will be developed and used to determine the occurrence of these antigens in patients with breast cancer and other types of cancer. 2) Human breast adenocarcinomas will be examined for the presence of specific biohemical markers that might be expected to be present on the basis of previous observations. These include viral markers and differentiation antigens. These compounds will be identified by immunofluorescence, quantified by radioimmunoassay, and isolated by immunospecific affinity chromatography. The physiochemical properties, including carbohydrate and amino acid compositions, will be compared with those of comparable glycoproteins isolated from normal tissues.

Progress: Extracts of human breast adenocarcinomas have been prepared by mild trypsinization. These were separated into three major fractions by gel filtration on Bio Gel P-100. The fraction that eluted in the void volume was further fractionated on Sepharose 4B. The elution patterns were compared with those obtained from similar extracts of fibroadenomas and other benign cysts excised at surgery from human breasts. Antibody against the trypsin extracts are being prepared in rabbits. After absorption with pools of benign cysts excised from breasts at surgery, the fractions obtained from Sepharose 4B will be examined for tumor specific antigenicity. The search for known glycoproteins that may be abnormally present in adenocarcinomas of the breast has led to the identification of a virus-related antigen.

Significance to Cancer Research: (NCP Objective 3 Approach 5)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $125,500 Site Visit Date: 4/20/77

CONTRACT RESEARCH SUMMARY

Title: Microcirculation and Molecular Transport in Mammary Carcinomas

Principal Investigators: Dr. Joseph F. Gross   Dr. Marcos Intaglietta
Name/Address: University of Arizona   University of California
Performing Organization: Tuscon, AZ   San Diego, CA

Contract Number: N01-CB-63981
Starting Date: 6/14/76   Expiration Date: 6/13/79

Goal: To study local blood velocity and pressure and quantify movement of materials across endothelium of single vessels into extravascular tissue in tumor microcirculation.

Approach: Fit Fischer 344 rats and nude-athymic mice with modified algire access chamber containing mammary adenocarcinoma to analyze microcirculatory parameters. Obtain quantitative morphologic data by planimetric techniques. Determine blood flow by measuring velocity of RBCs using dual slit technique. Determine capillary pressure by micropipette technique. Characterize diffusion and transport in extravascular compartments by analyzing extravasation of dyes injected intravenously. Develop analytical models to characterize microcirculation of tumor systems.

Progress: A new ultra-light weight aluminum chamber has these features: (1) fixed internal chamber spacing of 100 μ to achieve constant chamber environment for all preparations and experiments; (2) rigid metallic design to avoid instability during measurement of blood perfusion inherent in prior plastic chambers; (3) removable glass window to permit tissue access for sequential pressure and transport measurements; and (4) observed reduction of trauma due to surgery. A small chamber was built for the nude mouse with several preliminary implantations. The human mammary adenocarcinoma tumor has been established in our nude mouse colony. Sterile surgery and histologic tissue verification have been established in the preparation procedure. Daily photographic observations provided initial descriptions of morphological development of control and tumor preparations. Flow patterns in the microvasculature were analyzed up to 18 days from the time of installation. Similar observations were made after the implantation of a 0.1 mm^3 tissue fragment of mammary adenocarcinoma. All observations were carried out under light Pentobarbital anesthesia for 1 hour. Flow velocity of control preparations tended to increase continuosly during the period the vessels remained visible in the chamber window. The number of functioning capillaries and flow velocity in them increased on an average from 0.5 to 0.8 mm/sec over an 18-day period. Tumor preparations where the implantation was made 5-10 days after the installation of the chamber showed a progressive slowing of blood flow velocity in the region where there was active tumor proliferation, with eventual stagnation. Petechial hemmorrhages usually developed in those capillaries where flow resumed after stasis.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S., Richard Knazek, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $99,000   Site Visit Date: 2/5/76

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Cultures of Normal Mammary Epithelial Cells

Principal Investigator: Dr. Gordon H. Sato
Name/Address: University of California at San Diego
Performing Organization: La Jolla, CA

Contract Number: N01-CB-74188
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To isolate and maintain growth of pure normal mammary epithelial cells in culture.

Approach: Determine the hormonal and nutritional requirements of established animal and human mammary tumor cell lines. Proceed with similar studies on primary cultures of human mammary tumors obtained from autopsies and mastectomies. Determine the hormonal content, blood serum components, and growth factors (ovarian, nerve, epidermal, etc.) to prepare a defined medium to maintain growth and passage of normal mammary epithelial cell lines.

Progress: MCF-7 cells were plated for experiments in a 1:1 mixture of F12 and DME with a concentration of FCS which was limiting for growth. Hormones and growth factors were tested for their ability to stimulate cell division. Positive results were obtained with the following factors (lowest effective concentration tested given in parentheses): estradiol (10^{-9} M); dihydrotestosterone (10^{-8} M); insulin (0.05 μg/ml); EGf (4 ng/ml); fetuin 0.5 mg/ml); crude submaxillary extract 1 μg/ml); and partially purified urokinase. Conditioned media from RF1, GH_3, and BRL cell lines were also stimulatory. No stimulation was seen in the following: progesterone, hydrocortisone, aldosterone, prolactin, T_3, FGF, FSH, GH, LH, TSH, ACTH, NGF, TRH, PTH, LHRH, relaxin, thrombin, or putrescine. Best results were obtained in serum-free medium containing 2 μg/ml insulin, 4 μg/ml transferrin, 4×10^{-7} M dihydrotestosterone, 10^{-8} M estradiol, 40 ng/ml EGF, and 20% BRL conditioned medium. After a 2-day lag, cells in hormone-supplemented medium grew with a doubling time of 37 hours. Doubling time for cells in medium with 10% FCS was 35 hours. After 6 days, cell number in hormone supplemented serum-free medium was 65% of that in medium with 10% serum. Experiments are also in progress with primary cultures derived from a transplantable, estrogen-dependent human breast tumor line carried in nude mice. This tumor has not previously been grown _in vitro_.

Significance to Cancer Research: (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $61,600

CONTRACT RESEARCH SUMMARY

Title: Effects on Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Dr. Frank J. Talamantes, Jr.
Name/Address University of California
Performing Organization: Santa Cruz, CA

Contract Number: NO1-CB-74091
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: Determine the effects of perinatal exposure to exogenous estrogens and progestins, singly and in combination, on mammary gland noduligenesis and tumorigenesis in female mice.

Approach: C3H (MTV+, NIV+) and C3Hf (MTV-, NIV+) female mice will be treated perinatally with diethylstilbestrol (DES), 17 α-hydroxprogesterone caproate (HPC), or Ovral. The incidence, types, and age of appearance of mammary gland dysplasias will be recorded to determine if noduligenesis and tumorigenesis depend on: (1) the type and dose of hormone; (2) direct action of hormeone on the gland or on alteration of systemic factors. Changes in mammary sensitivity to an oral contraceptive and a chemical carcinogen after perinatal hormone exposure and the effects of hormone administration to lactating mothers on nonduligenesis and tumorigenesis in female offspring will also be determined.

Progress: Prenatal treatment: Primiparous females received daily sc injections of one of the following on days 10-17 of pregnancy: (1) 0.03 μg/day or 0.1 μg/day DES; (2) 15 μg/day or 150 μg/day HPC; (3) 25 ng/day ethinyl estradiol (EE_2) + 250 ng/day norgestrel (N) or 2.5 ng/day EE_2 + 25 ng/day N: or (4) sesame oil. Neonatal treatment: Female offspring of untreated primiparous mothers received daily sc injection of one of following on days 1-5 of life: (1) 0.001 μg/day, 0.1 μg/day, or 2.5 μg/day DES: (2) 15 μg/day or 150 μg/day HPC: (3) 25 ng/day EE_2 + 250 ng/day N or 0.25 ng/day EE_2 + ng/day N; monthly for 12 months for C3H animals and 18 months for C3Hf animals. Mammary gland whole mounts are being examined histologically.

Significance to Cancer Research: (NCP Objective 1 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $95,000 Site Visit Date: 4/22/77

CONTRACT RESEARCH SUMMARY

Title: Effect of Mammary Carcinoma Cells on the Vascular Endothelium

Principal Investigator: Name/Address Performing Organization:	Dr. Richard R. Lindquist University of Connecticut Farmington, CN

Contract Number: N01-CB-74089
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To elucidate the cancer cell-endothelium interaction and the effect cancer cells have on the vascular endothelium.

Approach: Human mammary cancer cells in co-culture with endothelial cells will be the model in studies to determine: (a) cancer cell-endothelial interactions using time-lapse phase contrast microscopy and electron microscopy; (b) interacting forces involved; (c) influence of various blood elements and cellular organelles on strength of the interactions; (d) cancer cell inflicted damage to endothelium; (e) cancer cell induced directional proliferation of endothelial cells. Human cells will be obtained from the Cell Bank (BCTF) and endothelial cells will be isolated from umbilical cords.

Progress: The interaction between mammary cancer cells and vascular endothelium was observed *in vitro* with the aid of scanning electron microscopy. Within 10 minutes of adding cancer cells to vascular enothelial organ cultures, cancer cell-endothelium interactions occurred and initial contact was made by long slender cytoplasmic projections (filopodia) from the cancer cells. Once the filopodia make initial contact, it is maintained by broad cytoplasmic projections. Because both cancer cells and endothelial cells have negative Zeta potentials and thus are mutually repulsive, the filopodia may be a mechanism for overcoming the electrostatic barrier between the two cells. In order to examine this possibility further, filopodia formation was inhibited by treating the cancer cells with cytochalsins, which interfere with microfilaments which are necessary for filopodia formation. Cytochalsin B (5 μg/ml) treated cancer cells have difficulty in establishing contacts with endothelial cells when compared to controls (113 ± 11 ± yrs 476 ± 31/mm^2). Thus, filopodia appear necessary for establishing optimum contact with the vascular endothelium. Further studies are planned to be sure that filopodia formation is not an epiphenomenon.

Significance to Cancer Research: (NCP Objective 4 Approach 3)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $68,700 Site Visit Date: 4/21/78

CONTRACT RESEARCH SUMMARY

Title: Osteotropism of Mammary Carcinoma Metastasis

Principal Investigator: Dr. Gregory R. Mundy
Name/Address University of Connecticut Health Center
Performing Organization: Farmington, CT

Contract Number: N01-CB-63980
Starting Date: 6/21/76 Expiration Date: 6/20/79

Goal: To identify the mechanisms by which bone is altered in breast cancer.

Approach: (1) Determine the cellular mechanism of bone destruction in metastatic breast cancer. (2) Determine the hormonal factors responsible for hypercalcemia and/or bone metastasis in breast cancer. (3) Characterize the effects of these factors on bone and identify ways in which those effects can be prevented or opposed. *In vitro* and *in vivo* systems of human and animal models used.

Progress: 1) The human breast cancer cell line MCF-7 has been found to have the ability to resorb bone *in vitro* directly independent of osteoclast-mediated mechanisms. This has been demonstrated by culture of MCF-7 cells on devitalized fetal rat long bones and by application of MCG-7 supernates to live bones co-cultured with cortisol (10^{-6}M) and phosphate (6mM), both of which inhibit osteoclast activity. The molecular mechanisms by which these breast cancer cells resorb bones is now under study.

2) Monocytes and macrophages are often found adjacent to breast cancer metastases. Recently, we found that these cells also directly resorb devitalized bone *in vitro* (Science 196:1109, 1977), which may explain some of the bone resorption which occurs in metastatic breast cancer. The mechanism of monocyte-bone resorption is also under study.

3) Prostaglandins are potent stimulators of bone resorption *in vitro* and have been implicated in the hypercalcemia of malignancy. We extended our earlier observations on PGE-mediated bone resorption by examining the effects of prostaglandin endoperoxides and other metabolites on bone resorption *in vitro* (Nature 267:5611, 1977). The endoperoxides produced rapid, but transient bone resorption, but the more stable dihydro metabolites were almost as potent as the parent PGE_1 and PGE_2. The 15-keto-13, 14-dihydro metabolites were much less potent. These studies are necessary to determine which particular products of prostaglandin metabolism are responsible for bone resorption in neoplastic disease.

Significance to Cancer Research: (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $109,300 Site Visit Date: 2/19/76

CONTRACT RESEARCH SUMMARY

Title: Structure Function Relationship of Prolactin Interactions in Mammary Gland Cells

Principal Investigator:	Dr. Kurt E. Ebner
Name/Address	University of Kansas Medical Center
Performing Organization:	Kansas City, KS

Contract Number: N01-CB-63985
Starting Date: 6/14/76 Expiration Date: 6/13/79

Goal: To modify ovine prolactin and determine the critical amino acids involved in binding of the hormones to membranes, activation of cyclases or kinase and stimulation of α-lactalbumin formation.

Approach: Modify prolactin chemically by using mercaptoethanol, N-ethylmaleimide, iodoacetamide, cyanogen bromide, tetranitromethane, and carbodiimide. Use trypsin, chymotrypsin, plasmin, pepsin, and leucine amino acid peptidase for enzyme modification of prolactin. Modifications will include: tyrosine, tryptophan, lysine, methionine, histidine, carboxyl groups, and reduction of disulfides. Prolactin activity will be assayed by binding to mammary particles, ability to activate cyclases or protein kinase in mammary plasma membranes, and stimulate α-lactalbumin synthesis in mid-pregnant mammary explants.

Progress: A radioimmune assay for mouse α-lactalbumin was developed using ^{125}I-α-lactalbumin labelled with lactoperoxidase. The effective standard curve is from 0-30 ng. Explants from mid-pregnant mouse mammary tissue form α-lactalbumin in response to prolactin when in the presence of insulin and hydrocortisone. The addition of thyroxine often causes a stimulation of α-lactalbumin synthesis.

Efforts are being continued on the cleavage of prolactin by chemical and enzymatic means. Prolactin is resistant to clostripain, elastase, and thrombin. Inter disulfide dimers of prolactin are inactive, but can be reduced to generate active monomers. Denatured and reduced prolactin reforms the correct disulfides very rapidly. Oxidation of methionines alone does not reduce binding. The oxidation of tryptophan reduced binding by two orders of magnitude ($K_d = 3 \times 10^{-7}$ M.) Combined oxidation of methionine and tryptophan drastically reduced binding to receptor.

Significance to Cancer Research: (NCP Objective 3 Approach 1)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $86,200 Site Visit Date 2/2/76

CONTRACT RESEARCH SUMMARY

Title: Purification of Estrogen Receptor in Tangible Amounts

Principal Investigator: Dr. Giovanni A. Puca
Name/Address University of Naples
Performing Organization: Naples, Italy

Contract Number: N01-CB-64074
Starting Date: 9/30/76 Expiration Date: 5/29/79

Goal: To isolate and purify the "native" form of estrogen receptor in milligram quantities.

Approach: Carry out large scale tissue disintegration and centrifugation for isolation of estrogen receptor from calves' uteri. Purify the "native" form of estradiol receptor by a combination of affinity chromatography on new adsorbents and conventional separation methods.

Progress: A new property which is peculiar to the "native" form of the estradiol receptor, i.e. its ability to interact specifically with heparin bound covalently to Sepharose, permits the purification of this form of the receptor. The receptor is eluted from the agarose with heparin in solution. Heparin impedes aggregation that until now blunted all attempts at its purification. This first heparin-agarose step can allow in few hours at least a 100-fold purification from cytosol. Purification to homogeneity was accomplished in a small scale experiment, after chromatography on columns of heparin-agarose, Sephandex G-200 and DEAE-cellulose, followed by two more Sephadex G-200 columns. The purified molecule is a single polypeptide of 69,000 MW by polyacrylamide gel electrophoresis in SDS. The sedimentation coefficient on sucrose gradients is 4.3 S, the Strokes radius from gel filtration is 36.5 Å, and the isoelectric point is 6.4. The purified $[^3H]$ estradiol-receptor complex exchanges the radioactive hormone only with estradiol or other estrogenic steroids, but not with testosterone, 5α-dihydrotesterone, and progesterone.

Large-scale experiments are at the moment hampered by the uterine supply. In fact, uteri from local slaughterhouses show a very low estradiol binding activity, and must be checked singly. We are trying to obtain uteri from other countries. Uteri can be stored in liquid nitrogen or at -80°, but they lose binding capacity when stored for a while at -20°.

Significance to Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: Dr. William H. Pearlman
Name/Address University of North Carolina School of Medicine
Performing Organization: Chapel Hill, NC

Contract Number: N01-CB-53905
Starting Date: 4/1/75 Expiration Date: 9/30/78

Goal: (1) To identify specific steroid-binding proteins in normal breast secretions, particularly in human colostrum during advancing pregnancy. (2) To determine the levels of the steroid-binding proteins in human and rat whey, and the influence of multiple endogenous and exogenous hormones on these levels. (3) To investigate the biological significance of the whey steroid-binding proteins.

Approach: (1) Development of a complementary method (charcoal-dextran) for the estimation and partial physiocochemical characterization of the whey steroid-binding proteins. (2) Correlation of changing endocrine status, i.e. known alterations in multiple hormone levels during advancing pregnancy, parturient and postpartum periods in the human) with changing levels of the steroid-binding proteins in whey and coincidental serum; similar studies on lactating rats following administration of estrogen or glucocorticoids. (3) The influence of the steroid-binding properties on the *in vitro* uptake and metabolism of tritium-labeled glucoroticoids by mammary alveolar cells.

Progress: (1) A testosterone-binding protein (TBP) together with a corticosteroid-binding protein (CBP) were detected in the whey of human colostrum and estimated by a charcoal-dextran method; the levels were highest during the prepartum period. (2) The charcoal-dextran procedure allowed determination of the half-time (t 1/2) for dissociation of the steroid-protein complexes; the t 1/2 values for whey TBP and CBP at 0° were the same as those for serume TeBG and CBG, respectively. (3) The levels of TBP and CBP in whey reflected of those of TeBG and CBG in coincidental serum, suggesting that the steroid-binding proteins in whey come from the plasma. (4) Mammary alveolar cells (isolated as intact acini from lactating rats) extensively metabolized ^{3}H-corticosterone to a nonpolar radiometabolite that accumulated in the nuclear fraction in amounts larger than those of ^{3}H-corticosterone. Identification of radiometabolite is in progress; the radiometabolite resists periodate oxidation indicating that the C17-ketol side chain is either blocked or has been eliminated. This is a novel finding of potential importance because the formation of a more active form of the hormone is suggested. Investigation, in turn, of the effect of the corticosteroid-binding proteins on mammary metabolism of glucocorticoids in this system would contribute to our understanding of mammary responsiveness.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 7/24/74

CONTRACT RESEARCH SUMMARY

Title: Methylation of Transfer RNA in Normal and Neoplastic Mammary Tissue

Principal Investigator:	Dr. Phoebe S. Leboy
Name/Address	University of Pennsylvania
Performing Organization:	Philadelphia, PA

Contract Number: N01-CB-74108
Starting Date: 8/15/77 Expiration Date: 8/14/79

Goal: To examine tRNA methylation in normal mammary gland and DMBA-induced hormone responsive and nonresponsive tumors in the rat.

Approach: Compare the level of methyltransferase activity in normal quiescent, late pregnant, and lactating mammary tissue and DMBA-induced mammary tumors. Compare patterns of tRNA methyltransferase activities in hormone responsive and nonresponsive tumors with those from non-neoplastic mammary tissue. Purify individual tRNA methyltransferases from mammary tumors and corresponding enzymes from normal mammary gland,and compare their biochemical properties. Test metnyltransferase inhibitors *in vitro* and *in vivo* to determine the effect on cell or tumor growth.

Progress: The total tRNA methyltransferase activity/mg tissue protein in tumor extracts is three times higher than in normal mammary extracts and about 40% higher than in extracts from late-pregnant mammary gland; however, the relative distribution of individual methyltransferases is the same in all three extracts. The extent of methylation of a limiting amount of tRNA substrate with normal mammary extracts is only 40-70% of that achieved with tumor enzymes. When analyzed with purified tRNSs, this difference appears attributable to an increased capacity of tumor extracts to approach the theoretical maximum of 1 methyl group/site/tRNA molecule in the formation of 5-methyl cytosine and 2-methylguanine.

Significance to Cancer Research: (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $99,600 Site Visit Date: 4/18/77

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine-Induced Breast Regression

Principal Investigator: Dr. William L. McGuire
Name/Address University of Texas Health Science Center
Performing Organization: San Antonio, TX

Contract Number: N01-CB-23862
Starting Date: 6/1/72 Expiration Date: 5/31/79

Goal: To obtain new information of the hormonal control of breast tumor growth and to identify those breast cancer patients whose disease will respond to hormonal manipulation.

Approach: Investigate the sequence of events from initial estrogen binding to receptor sites to nuclear localization of receptor complex and estrogen stimulation of progesterone receptor complex and estrogen stimulation of progesterone receptor synthesis.

Progress: Regardless of tumor size or location, axillary node status, or age, patients with estrogen receptor negative (ER-) primary tumors recur earlier than patients with ER+ tumors. Similarly, survival is adversely affected.

Approximately 150 human breast tumors have been assayed for ER and evaluated for clinical response to endocrine therapies. Tumors without ER have a low response rate (6%), whereas ER positive tumors had 60% objective regression. Tumors with very high ER values had the highest remission rate.

New assays are currently being developed to measure nuclear bound ER. Previous efforts were unsuccessful because of nuclear protease activity that promptly degrades nuclear ER. Results with new assay avoid protease problem. An unexpected finding of potential major significance is presence of nuclear ER without attached steroid in certain tumors. We speculate that uncharged nuclear ER may be stimulating replication, perhaps explaining lack of tumor regression following ablative procedures. Antiestrogens may be the choice of therapy in these special tumors.

We have chosen progesterone receptor (PgR) synthesis as a new biochemical marker of an intact estrogen response system. Estrogen-priming in DMBA rat mammary tumors and MCF-7 human breast cancer cell line results in dramatic induction of PgR. In metastatic human breast tumors, the presence of PgR directly correlates with the level of ER. In patients, ER and PgR correlate well with response to therapy. ER-, Pgr-0%, ER-, PgR+ no patients; ER+, PgR-41%, ER+, PgR+81%. The presence of PgR in ER+ tumors gives considerable confidence in predicting response to therapy.

Significance to Cancer Research: (NCP Objective 5 Approach 6)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $125,000 Site Visit Date: 2/24/72

CONTRACT RESEARCH SUMMARY

Title: Alteration of Mammary Neoplastic Cells by Manipulation of Cellular Environment

Principal Investigator: Name/Address Performing Organization:	Dr. Creed W. Abell University of Texas Medical Branch Galveston, TX

Contract Number: N01-CB-43906
Starting Date: 6/10/74 Expiration Date: 6/9/78

Goal: To manipulate the cellular environment *in vivo* and *in vitro* in order to limit preferentially the growth of neoplastic cells of mammary origin.

Approach: To evaluate the effects of phenylalanine ammonia-lyase (PAL) and phenylalanine restriction on mammary tumors in mice and rats, and on human breast tumors growing in athymic mice. The host will be immunosuppressed to decrease the rate of clearance of PAL, particularly after repeated exposure.

Progress: PAL, isolated from the yeast *Rhodotorula glutinis*, has been purified to homogeneity and evaluated for its therapeutic efficacy both alone and in combination with phenylaline mustard (PAM) against BW10232 and 15091A mammary tumors in mice. Repeated injections of PAL resulted in the formation of circulating antibodies (anti-PAL) to PAL which were demonstrated by passive hemagglutination and by radioimmunoassay. Temporary immunosuppression to PAL was achieved by sublethal total body radiation. In contrast, administration of a single dose (180 mg/kg body weight) of cyclophosphamide (CPA) with PAL resulted in tolerance of the host to repeated injections of PAL (14 times) for 8 months. During this period of time, the half-life of the enzyme remained the same as that determined after a single injection (approximately 22 hours) and titers of anti-PAL were undetectable in CPA-PAL treated mice. The administration of PAM with PAL in immunosuppressed tumor-bearing mice resulted in significant inhibition of tumor growth, but host toxicity occurred presumably as a result of phenylalanine deprivation. Studies are currently being conducted to reduce toxicity through dietary supplementation with phenylpyruvate in attempts to inhibit preferentially tumor growth through PAL therapy.

Significance to Cancer Research: (NCP Objective 6 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 3/14/74

CONTRACT RESEARCH SUMMARY

Title: Differentiation of Mammary Epithelial Cells

Principal Investigator:	Dr. Howard L. Hosick
Name/Address	Washington State University
Performing Organization:	Pullman, WA

Contract Number: N01-CB-63986
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To study how several mesenchymal cell types in the adult mouse mammary gland interact with mammary epithelium to regulate its development.

Approach: Isolate mammary epithelial cells from C3H mice in midpregnant state. Grow these cells as monolayers in culture dishes and on various permeable substrata. Test the responses of the epithelium, under various endocrine conditions, to other mammary cell types and their products. The emphasis is on interactions with adipose cells and fibroblasts. Responses of epithelium, to fatty acids will be measured and metabolism of exogenously supplied lipids will be analyzed. Responses of normal, preneoplastic, and neoplastic cells will be compared. Undertake studies for defining conditions for growth and passage of primary cultures of normal epithelial cells.

Progress: Unilamellar liposomes containing free fatty acids have been prepared and used to insert the ffa directly into mammary cells in culture. The rate of uptake by tumor cells is about twice that by normal cells. Exogenously supplied fatty acids are rapidly metabolized by the epithelial cells, mostly to triglycerides and phospholipids. Non-epithelial mammary cells are also sensitive to the lipid composition of culture medium, and can be selectively suppressed by manipulating this composition. Cells are being grown on permeable substrata (artificial capillaries, collagen gels, Sephadex beads in suspension) so as to make their basal surfaces accessible to lipids and other precursors, and hormones. Mammary epithelium also responds to an extract of embryonic mesenchyme. This extract promotes differentiation of normal mammary cells as manifested by production of lactose, but only in the presence of insulin, hydrocortisone, and prolactin. Preneoplastic cells (hyperplastic alveolar nodules) are not stimulated to differentiation of cells from spontaneous mammary tumors. The same studies will be repeated using human mammary cells obtained from breast fluids.

Significance to Cancer Research: (NCP Objective 3 Approach 3,5)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds; $55,000 Site Visit Date: 2/4/76

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of Mammary Cell Surface

Principal Investigator: Dr. Clayton A. Buck
Name/Address Wistar Institute
Performing Organization: Philadelphia, PA

Contract Number: N01-CB-64069
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: To determine the synthesis and fate of mammary tumor glycoproteins.

Approach: Glycoproteins will be derived from mouse mammary tumor systems and normal mammary tissue labeled in situ or following growth in culture with ^{3}H- or ^{14}C-labeled sugars. Future studies will use human mammary cell lines from the Cell Culture Bank and surgical specimens. Membrane-bound and shed glycoproteins will be analyzed by polyacrylamide gel electrophoresis. Antisera will be prepared against material shed from tumor and control cells. The antisera will be used to identify and isolate membrane glycoproteins shed from tumor cells. Lectin and immunoglobulin affinity columns will be used in the purification of glycoproteins. Turnover of specific membrane glycoproteins will be followed using specific antisera and affinity chromatography.

Progress: 1) Kinetics and glycoprotein shedding: Cells were pre-labeled with ^{3}H amino acids and ^{14}C-fucose. The rate of loss of isotope was followed using 3 murine cell lines and 3 humor tumor cell lines. In every case over half the ^{3}H or ^{14}C isotope was shed into the medium. The half-life was between 18 and 24 hours. The rate of shedding was independent of growth rate or the presence of serum. With one exception, between 50% and 70% of the shed ^{14}C-fucose was in a nondialyzable form, while only 5% of the shed amino acids were nondialyzable. 2) Polyacrylamide gel electrophoresis of shed glyoproteins: Glycoproteins shed from both mouse and human cells revealed no outstanding glycoproteins common to either mouse tumors or human tumors. 3) Immunological Studies: Antisera have been prepared in both goats and rabbits against material shed from 2 mouse tumor cell lines, 1 "normal" mouse cell line and from primary mouse mammary cells. In addition, antisera have been prepared against similar material shed from human tumor cell lines. The antisera react in Ochterloney immunodiffusion tests only with material shed from homologous tumor cell. There is no evidence of a common mammary tumor specific antibody. These antisera react in indirect immunoprecipitation with at least 20% of the fucose-containing glycoproteins from homologous cells. Electrophoretic analysis of this material is underway.

Significance to Cancer Research: (NCP Objective 5 Approach 3,4)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $89,300 Site Visit Date: 4/27/76

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Name/Address	Dr. Grant Fairbanks Worcester Foundation for Experimental Biology
Performing Organization:	Shrewsbury, MA

Contract Number: N01-CB-33908
Starting Date: 6/1/73 Expiration Date: 2/28/79

Goal: Development of new methods for plasma membrane isolation and characterization applicable to normal and neoplastic mammary cells.

Approach: Membrane modification reagents and activated beads will be used to isolate plasma membrane fragments from ascites cells of the metastatic 13762 rat mammary andenocarcinoma. Macromolecular organization at the extracellular and cytoplasmic faces of the membranes will be analyzed and combined application of selective radiolabeling, protein cross-linking and enzymatic dissection.

Progress: In the first radiosynthesis of a cleavable protein cross-linking reagent, ^{35}S-dithiobis (succinimidyl propionate) (^{35}S-DTSP) was prepared at 20 mCi/mmole. DTSP combines high reactivity against amino groups with long lifetime in an aqueous solution. Under mild conditions (e.g., 1-5 minutes at o°C, pH 7, with 10^{-5}-10^{-5} M DTSP), it is an efficient probe of protein-protein interaction in membranes and other extended structures. We found ribonucleotide homoplymers unsuited as affinity reagents for membrane isolation, but demonstrated specific binding of ^{3}H-poly(U) to surface proteins on 13672 cells. Poly(U) binding may probe nucleoside transport carrier sites structurally and topographically distinct from lectin receptors. Using polylysinated beads, we isolate 13672 membrane fractions enriched ten- to fifteen-fold in plasma membrane markers. Higher enrichment is unlikely because cells initially bind to beads via tips of microvilli. We are comparing membrane composition and organization in this specialized subfraction to that of the 13672 cell surface as a whole. Surface radioiodination glycoproteins on 137672 cells. Two-dimensional electrophoretic analysis reveals extensive cross-linking of unlabeled, internal proteins, but no cross-linking of ^{125}I-labeled surface proteins. The attachment and organization of the most accessible elements ("surface coat") apparently does not involve extensive specific protein-protein interactions.

Significance to Cancer Research: (NCP Objective 3 Approach 5)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $59,700 Site Visit Date: 5/3/73

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator: Dr. Heinz Furthmayr
Name/Address: Yale University
Performing Organization: New Haven, CT

Contract Number: N01-CB-84225
Starting Date: Expiration Date:

Goal: To prepare immunochemically defined antibody reagents to collagen of types I, II, III, IV (basement membrane), AB (fetal collagen), procollagens of types I and III, and mouse collagen of types I, III, and IV.

Approach: Studies include: (1) isolation and purification of collagens from calf, human, and mouse tissues and isolation of peptide fragments; (2) immunization of rabbits, guinea pigs, and sheep; (3) serological screening of antisera during immunization and after final bleeding; (4) purification and isolation of antibodies; specific for each collagen and/or peptide fragments; (5) characterization of the isolated antibodies; and (6) storage and distribution of the purified antibodies to other investigators.

Progress: New Contract

Significance to Cancer Research: (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 4/28/78

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. Lewis M. Schiffer
Name/Address Allegheny Singer Research Corp.
Performing Organization: Pittsburgh, PA

Contract Number: N01-CB-43899
Starting Date: 2/1/74 Expiration Date: 1/31/79

Goal: To develop and exploit methods of rapidly determining cell kinetic parameters of individual human breast cancers so that this information may potentially be used in the study of the natural history and treatment of these tumors.

Approach: Three cell kinetic parameters of human and non-human solid breast tumors are measured directly by tumor imprints and mechanical disaggregation of tissue followed by *in vitro* incubation with isotopically labeled thymidine. These parameters are 3H-TdR labeling index, DNA synthesis time, and PDP index (which estimates growth fraction). Potential doubling time and cell cycle time can be calculated from these values. Other techniques, such as autoradiographic grain counting and freezing to transport specimens, are being developed to pursue the goal of clinical utility. A data base of human breast tissue, both benign and malignant, has been developed and correlated with clinical parameters.

Progress: The results of the human breast cancer data are presented here. The mean 3H-TdR LI in primary tumors was 0.056 with a median of 0.047 and range of 0.005 to 0.343. Metastatic lesions had a mean of 0.086, median of 0.078, and range of 0.037 to 0.142. The mean PDP index (growth fraction estimate) of primaries was 0.286, and median 0.226. The mean DNA synthesis time was relatively invariant at 18.4 and 19.4 hours respectively. Calculations for potential doubling time showed a mean of 13.6 days and a median of 10.2 days for primary tumors and 7.6 and 6.4 days for metastatic tumors. The mean calculated cell cycle time for primary tumors was 120.6 and median 93.6 hours. The respective median values for metastatic tumors were 64.5 and 68.4 hours. Of interest was the great heterogeneity of the values. Other studies on animal tumor models and clinical use of these results are continuing.

Significance for Cancer Research: (NCP Objectives 5 Approach 6)

Program Officers: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $116,000 Site Visit Date: 10/31/74

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Name/Address Performing Organization:	Dr. Lewis M. Schiffer Allegheny-Singer Research Corp. Pittsburgh, PA

Contract Number: N01-CB-74083
Starting Date: 7/1/77 Expiration Date: 6/30/79

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The experiments on two animal tumor models, the DMBA-induced rat mammary tumor and the C3H/He mouse spontaneous mammary tumor, will test the correlation of unperturbed tumor cell kinetics and tumor response in individual animals to chemotherapeutic agents. The hypothesis to be tested is that the highly variable cell kinetic parameters are related to the drug responsiveness of tumors. The cell kinetic studies will include tritiated thymidine labeling index, DNA synthesis time, and tumor growth fraction estimation via primer-available DNA polymerase assay.

Progress: Over 100 DMBA tumors have been analyzed for the basic technical studies. Cell kinetic comparison of excisional and needle biopsy shows no significant differences. Alterations in cell kinetics become normalized at 72 hours. The mean tumor doubling time was 40.7, and median 19.9 days with a log-normal distribution. Well differentiated tumors had lower 3H-TdR and PDP indices than did poorly differentiated tumors. There is a suggestion of a subpopulation with very high proliferative values. There was no significant correlation of cell kinetic values and tumor burden. The overall mean 3H-TdR LI was 0.061 with a range of .022 to .168; PDP, .211 (.044 to .653); TS, 9.4 hr (7.2 to 13.5); TP 133.9 hr (36.5 to 321.3); amd TC 38.9 hr (11 to 144.3). The drug studies are underway with 80 mgm/kg cyclophosphamide showing an average regression of 53% in tumor volume with a nadir at 8.7 days. 100 mgm 5-FU is showing an average tumor volume decrease of 59% at a nadir of 6.8 days.

Significance for Cancer Research: (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary L. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $118,500 Site Visit Date: 1/26/77

CONTRACT RESEARCH SUMMARY

Title: Therapy for Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Name/Address Performing Organization:	Dr. Charles A. Hubay Case Western Reserve University Cleveland, OH

Contract Number: N01-CB-43990
Starting Date: 6/16/74 Expiration Date: 6/15/79

Goal: To test the effects of adding chemotherapy, anti-estrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer (Stage II-III).

Approach: Patients under age 76 who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, is on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments are (1) cyclophosphamide, methotrexate, 5-flurouracil (CMF); (2) cyclophosphamide, methotrexate, 5-fluorouracil, tamoxifen (CMFT) for 12 months; and (3) CMFT for 12 months plus BCG for 12 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: This study began in September 1974, and 255 patients have been enrolled. Of these, 76% had Er positive tumors (at least 3 femtomoles/mg. of tissue). In addition, more than 400 patients treated with mastectomy alone for Stage I breast cancer have been followed. Thirty-three of the study patients have had treatment failure, 19 were estrogen receptor positive and 14 were estrogen receptor negative. Patients with 4 or more metastatic acillary nodes have exhibited a failure rate three times that of patients with 1-3 involved nodes. No significant differences exist among the three treatment groups at this time.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $200,000 Site Visit Date: 4/1/74

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Name/Address Performing Organization:	Dr. Edward F. Scanlon Evanston Hospital Evanston, IL

Contract Number: N01-CB-53917
Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: To determine the effects of adding chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have metastatic axillary nodes or Stage III breast cancers are admitted to the study program. Stratification and randomization are carried out along with a balancing for prognostic factors according to the following variables: Primary tumor size, number of positive nodes, menstrual status, and unfavorable local signs. The statistical analysis involves sequential treatment assignment and is designed to provide the greatest balance between all groups assigned according to all available prognostic indicators. One of three treatment schedules is assigned to each of the patients in the study according to the above computer classifications: (1) Intermittent phenylalanine mustard (PAM); (2) 5-fluorouracil, cyclophosphamide, and prednisone in intermittent courses; and (3) the three-drug chemotherapy plus BCG inoculations. Two of the three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The statistical method of analysis is also identical with that now being used at the Mayo Clinic. The treatment courses will be given for one year or until there is evidence of relapse, whichever occurs first. Immunologic evaluation is done initially and every three months. Survival and recurrence-free intervals will be computed for each treatment group.

Progress: Since July 1975, the study has enrolled 141 patients. Entry to the PAM regimen was discontinued because of a disporportionately high tumor recurrence rate, 12 of 38, compared to four of 52 patients in each of the other two treatment arms. Fifteen of the 20 patients with recurrences had primary tumors larger than 3 cm., and 13 had four or more metastatic axillary nodes. The patients who received the polychemotherapy had a higher frequency of nausea, vomiting, diarrhea, alopecia, and stomatitis than those who received PAM; however, PAM results in more severe thrombocytopenia. Phytohemoglutination analysis and skin tests do not reveal any significant trends among treatment groups.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $99,400 Site Visit Date: 8/29/74

Title: Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: Name/Address Performing Organization:	Dr. Arthur E. Bogden Mason Research Institute Worcester, MA

Contract Number: N01-CB-43914
Starting Date: 11/1/73 Expiration Date: 10/31/78

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in six transplantable rat mammary tumor systems having a spectrum of histological, growth, and metastasizing characteristics. The highly metastatic 13762 mammary adenocarcinoma is used most extensively, and the R3230AC, SMT-2A 3M2N, DMBA#1, and DMBA#14 mammary tumors are used to test further and evaluate those drug and therapy combinations showing promising activity in the 13762 system. Chemotherapy assays versus subcutaneous grafts are made by initiating drug treatment on the day following implantation or on day 15 when tumors are well established. Metastasis assays combine surgical extirpation of tumors with the modalities of chemotherapy, x-irradiation and immunotherapy, alone and in various combinations. The parameters of immunosuppression and hormone radioimmunoassay are applied in studies requiring in-depth drug and therapy evaluation. Chemotherapeutic agents of current interest include phenylalanine mustard (PAM), adriamycin (ADR), cytoxan (CTX), 5-fluorouracil (5-FU), methotrexate (MTX), dibromodulcitol (DBD), hexa-methylmelamine (HMM), vincristine (VIN), and the nitrosoureas.

Progress: Studies with the 13762 mammary tumor predictive for those human breast cancers responsive to alkylating agents have shown that a regimen in which treatment initiated with a more effective agent (DED) was interrupted with a less effective agent (CTX) for a brief pulse at time of maximum remission was superior to continuous treatment with the more effective agent in terms of remission induction and percent "cures." Of various combinations of PAM, CTX, DBD, and HMM, the short pulse effect was characteristic only of the DBD and CTX combination. Discontinuing treatment for a short pulse had no beneficial effects. Administration of PAM + ADR to maximum remission followed by crossover to PAM + 5FU was more effective than the reverse, or treatment with only the PAM + ADR combination. Initial toxicity studies in preparation for utilizing a combination of an immunorestorative (Levamisole) and a macrophage activator (Staphylococcal Phage Lysate) as an immunotherapeutic modality indicate a lack of toxicity at effective dose levels. Endocrine effects of the above listed agents are being evaluated in acute and chronic regimens.

Significance for Cancer Research: (NCP Objective 6C Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $149,673 Site Visit Date: 3/22/73

CONTRACT RESEARCH SUMMARY

Title: Biomedical Computing Software Services

Principal Investigator:	Marlene Dunsmore
Name/Address	Mason Research Institute
Performing Organization:	Worcester, MA

Contract Number: N01-CB-74140
Starting Date: 3/21/77 Expiration Date: 3/20/79

Goal: To increase the usefulness of data produced in projects related to the treatment of human breast cancer.

Approach: A central data file will be set up for three areas of study: (1) surgical adjuvant therapy, (2) tumor markers, and (3) estrogen receptor assays. The file will allow comparison of the results from various studies and provide a data base from which material can be quickly and conveniently retrieved when desired for testing new ideas, identifying groups of patients suitable for more detailed study, and for preparing reports to the medical and general public. The file is not intended to duplicate those at individual institutions but to perform analyses that are not possible at the individual institutions.

Progress: The basic data collection and editing systems are operative. Clinical and laboratory information is abstracted and entered via a computer terminal into an on-line data collection system that edits for consistency upon entry. Estrogen receptor assay (ER) values and initial clinical data have been furnished by five institutions on almost 1500 patients. On 669 of these, at least one followup report has been submitted. Preliminary analyses of these data indicate a positive correlation of ER with length of disease free interval and age and negative correlation with grade of tumor and extensive lymph node metastases. Work has begun on the Biologic Tumor Markers Project. The data center maintains clinical history files associated with blood serum samples provided by three institutions and stored and inventoried at the Mayo Clinic.

Significance for Cancer Research: (NCP Objectives 6 Approach 5)

Project Officer: Mary E. Sears, M.D., Ihor Masny K. Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $91,168

CONTRACT RESEARCH SUMMARY

Title: Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator:	Dr. David L. Ahmann
Name/Address	Mayo Foundation
Performing Organization:	Rochester, MN

Contract Number: N01-CB-33899
Starting Date: 6/30/73 Expiration Date: 6/29/79

Goal: To assess the effects of surgical adjuvant chemotherapy using a multiple drug program: cyclophosphamide, 5-fluorouracil, and prednisone (CFP), versus CFP plus irradiation therapy versus a program using phenylalanine mustard alone.

Approach: Patients with unfavorable primary breast cancers (node +) are assigned to treatment programs involving mastectomy followed by multiple drug after chemotherapy (CFP), multiple drug chemotherapy (CFP) with postoperative irradiation, or single agent chemotherapy using phenylalanine mustard. After stratification according to menstrual category, tumor size and degree of involvement, each patient is randomly assigned to one of the three treatment programs as indicated above. The adjuvant chemotherapy is initiated two to four weeks postoperatively and given concomitantly with radiation therapy when this modality also is employed. Chemotherapy is terminated at the end of one year (10 courses) or upon the appearance of recurrent disease, whichever occurs first.

Progress: Of the 166 patients entered into the clinical trial, 37 (22 percent) have recurrent disease, 24 disseminated, and 13 local or regional. In the premenopausal patients, CFP with or without radiation therapy appears to be superior to phenylalanine mustard with respect to progression-free intervals. Longer followup will be needed to determine the effect of the treatments on permanent disease-free states and on survival. Toxicity has been mild to moderate. Myelosuppression occurs in most patients. Only about 25% of the premonopausal women have had significant menstrual disturbances, suggesting that both tumor kinetic factors and hormonal changes may exert influences.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Occult Breast Cancer Metastases in Axillary Lymph Nodes

Principal Investigator: Dr. Edward J. Wilkinson
Name/Address: The Medical College of Wisconsin
Performing Organization: Milwaukee, WI

Contract Number: N01-CB-74110
Starting Date: 7/1/77 Expiration Date: 6/30/79

Goal: To determine if the presence of occult lymph node metastases in axillary lymph nodes previously diagnosed as negative correlates with clinical recurrence of breast cancer.

Approach: The investigators will restudy comprehensively the lymph nodes removed from approximately 500 patients at the time of mastectomy for primary breast cancer. In all cases the initial routine histological examination of the nodes demonstrated no evidence of metastases. A pilot study on a subset will use serial sectioning of node material, and the best spacing between sequential subsets of these slides can be determined by re-examination of the subsets. Five- to ten-year followup information will be available on all patients. The study will consider the following factors in relation to their risk for clinical recurrence: primary tumor type, grade, and volume; node number removed, number positive and positive levels; metastatic volume; pretreatment and post-treatment records; age; and survival information.

Progress: To date, preliminary data have been obtained on 544 patients with 5 to 10 or more year followup. Complete data have been collected on 94 of these pateints as of September 20, 1977.
All necessary cases and blocks have been identified for the pilot study, and the serial sectioning of these cases is ongoing. Serial sectioning has been completed on 50% of the cases estimated in the pilot study. The paraffin blocks containing the unsectioned node tissue from 183 patients in the study have been obtained to date.
Time studies have been performed on sectioning of blocks and slide examination. Work sequencing has been arranged to conform with the analysis of these studies.

Significance for Cancer Research: (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 110,900 Site Visit Date: 3/1/77

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents for Breast Cancer Therapy

Principal Investigator: Name/Address Performing Organization:	Dr. Richard J. Santen Pennsylvania State University Milton S. Hershey Medical Center Hershey, PA

Contract Number: N01-CB-53851
Starting Date: 5/15/75 Expiration Date: 5/14/79

Goal: To proceduce suppression of adrenal function with aminoglutethimide and to compare the effects upon human breast cancer with those of adrenalectomy.

Approach: Female patients with inoperable, recurrent, or metastatic breast cancer are admitted to this study, which is expected to establish the optimal technique for aminoglutethimide (AG) blockage of adrenal steroid synthesis and glucocortiocoid suppression of the reflex rise in ACTH. The effects of AG on extra-adrenal estrogen production, ovarian function, and the hypothalamic and pituitary control of prolactin secretion are also being studied. The differential actions of the D-L racemic and the D form of AG will be established. Finally, the effects on human breast cancer of endocrine suppression by this noninvasive technique will be compared with those of surgical ablation in a randomized trial.

Progress: A total of 136 women with breast carcinoma have been entered. Steroid metabolic studies demonstrated that AG blocks estrogen synthesis at two sites, the adrenal and extra-adrenal peripheral tissues. The second site is important because the ovary continues to secrete estrogen precursors after the menopause and AG can inhibit estrogen production form this source. Consequently, estrone and estradiol levels are equally suppressed in surgically castrate or spontaneously menopausal women. Pharmacokinetic studies revealed that AG causes acceleration of its own metabolism since the basal drug half-life of 14 hr decreased to 6 hr during therapy ($p <.001$). Initial drug side effects were reduced by lowering AG dosage during the first 2 weeks of therapy to compensate for reduced metabolism. The D stereoisomer of AG has twice the potency of the DL racemic form and consequently is promising as a more effective agent. A fixed-dose combination of AG and hydrocortisone was established that effectively inhibits estrogen synthesis. Forty percent of postmenopausal women responded to AG with objective tumor regression. Patients are now being entered into protocols comparing surgical with medical adrenalectomy and medical adrenalectomy with tamoxifen.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $224,000 Site Visit Date: 3/7/75

CONTRACT RESEARCH SUMMARY

Title: Investigation of Steroid Sulfation and Estrogen Binding in Human Breast Cancer

Principal Investigator:	Dr. Thomas L. Dao
Name/Address	Health Research, Inc.
Performing Organization:	Roswell Park Memorial Institute Buffalo, NY

Contract Number: N01-CB-43900
Starting Date: 2/1/74 Expiration Date: 1/31/79

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels have been assayed on breast tissue obtained from approximately 300 patients in three institutions. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with histopathological parameters. The predictive potentials of ER and sulfation activity will be compared.

Progress: A total of 266 patients from three cooperating institutions have been entered into the study between March 1974 and June 1976, inclusive. The average length of followup is now approximately 20 months. A total of 25 patients were excluded for the following reasons: 1) the patients received treatment not described in the protocol; 2) they did not receive primary treatment as described in the protocol; 3) they were lost to followup. Based on an analysis of 205 patients from two institutions (the followup of 37 patients from the third institution is not complete and, therefore, is not included in this report), the data disclose: that 1) there was a 31% recurrence rate in patients with ER negative tumors as compared with a recurrence rate of 18% in patients with ER positive tumors; 2) in premenopausal women, the recurrence rate is 33% in ER negative patients and 25% in patients with ER positive tumors. In contrast, the recurrence rate in postmenopausal women is much higher in those with ER negative tumors than in those patients with ER positive tumors (30% vs 16%). 3) the data suggest that the recurrence rate is related to a low level of DHEAS, particularly in patients with no lymph node metastasis; thus, recurrence has occurred in 19% of the patients whose DHEAS levels are less than 25 pg/mg protein and in 11% of the patients whose tumors synthesize much higher levels of DHEAS ($>$ 25 pg/mg protein). Longer followup is needed to confirm or refute the prognostic significance of steroid sulfating and ER activity in the tumor.

Significance for Cancer Research: (NCP Objective 6 Approach 6,4)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $23,200

CONTRACT RESEARCH SUMMARY

Title: Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator:	Dr. Arthur H. Rosoff
Name/Address	Rush-Presbyterian-St. Luke's Medical Center
Performing Organization:	Chicago, IL
Contract Number: N01-CB-43991	
Starting Date: 6/17/74	Expiration Date: 6/16/78

Goal: To evaluate the relative merits of early versus delayed combination chemotherapy in patients with metastatic breast cancer known to have responded to surgical oophorectomy.

Approach: Premenopausal patients with metastatic progressing breast cancer are evaluated 12 weeks following surgical castration. Those patients who are showing no change or tumor regression (partial or complete response) are randomly assigned to the maintenance phase of either (1) observation or (2) cyclic treatment courses of cyclophosphamide, 5-fluorouracil, and methotrexate (CMF).

When relapse is documented in patients assigned to observation, CMF treatment will be started. The results of these studies are expected to compare regression rate, time to progression, and survival in these two groups of patients.

Progress: Ninety-eight (98) patients have been registered for the oophorectomy phase of the study. Thirty-two (32) have been randomized to the maintenance phase. Forty-four percent of the patients have achieved complete (4 patients, 6%), partial response (11 patients, 17%), or stable disease (13 patients, 21%), twelve weeks post oophorectomy.

For patients experiencing complete response to oophorectomy, there was no change in 2 of 2 on maintenance. Of seven patients experiencing partial response to oophorectomy, 2 became complete, 2 demonstrated no change, and 3 progressed with maintenance treatment. Of the 10 patients demonstrating no change following oophorectomy, 2 complete responses, 2 partial responses, 1 patient with stable disease, and 5 patients who progressed were noted.

Complete information on the outcome of the patients on the maintenance phase of this study is not yet available. The life table estimate of the overall median survival from the date of oophorectomy is 19 months with 45 of 66 patients still living. Since 16 of the 19 patients with evaluable maintenance case records were still living at the time of the last survival report, it is too early to estimate the median survival by maintenance regimen at this time.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II Carcinoma of the Breast

Principal Investigator:	Dr. Donald L. Morton
Name/Address	University of California
Performing Organization:	Los Angeles, CA

Contract Number: N01-CB-43917
Starting Date: 6/17/74 Expiration Date: 6/16/79

Goal: To evaluate the effectiveness of adding chemotherapy or chemoimmunotherapy stimulation to mastectomy for patients with Stage II carcinoma of the breast.

Approach: Patients with any number of positive axillary lymph nodes are eligible to enter this study, which randomly assigns one of the following treatment schedules: (1) cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); (2) CMF plus BCG; or (3) CMF plus BCG plus tumor cell vaccine. The tumor cell vaccine consists of irradiated allogeneic breast carcinoma cells grown in tissue culture. CMF is given in 12 cycles over 64 weeks. Immunotherapy is given for 114 weeks. Treatment failure consists of either metastases or development of a new carcinoma in the contralateral breast. The patients undergo evaluation of immunologic parameters before treatment begins and at intervals throughout the study.

Progress: One hundred twenty-five patients have been entered into the protocol since its inception in June 1974. Patients have been randomized into one of the three areas as follows: CMF, 31 patients; CMF + BCG, 54 patients; and CMF + BCG + TCV, 40 patients. The longest followup is 45 months. Twenty-three patients have developed metastases, seven occurring while on treatment. Three other patients have developed a second primary in the contralateral breast.

About 80% of patients have had serial testing of immunocompetence by measuring delayed hypersensitivity to DNCB and PPD. The increased reactivity to repeated challenges to DNCB continues to be less in breast cancer patients receiving CMF and BCG compared to a group of melanoma patients receiving only BCG.

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $125,000 Site Visit Date: 10/10/75

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Elwood V. Jensen University of Chicago Chicago, IL

Contract Number: N01-CB-43969
Starting Date: 6/16/66 Expiration Date: 6/15/79

Goal: To develop techniques which will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1966, Dr. Jensen has been studying estrogen receptors (ER) in human breast cancer tissues and the relationship between ER content and response to endocrine manipulative treatments. Because it now appears that patients whose breast cancers contain small or negligible amounts of ER have little if any chance of responding to endocrine therapy, whereas most but not all pateints with tumors containing substantial amounts of ER receive benefit from hormonal manipulation, attention is directed toward three principal objectives: (1) understanding of why some receptor-containing breast cancers do not respond; (2) development of a simple and specific radioimmunoassay for ER in breast cancers; and (3) evaluation of the ability of an ER assay on the primary tumors, carried out at the time of mastectomy, to predict subsequent response to endocrine therapy.

Progress: Of more than 1,200 patients whose primary and/or metastatic breast cancers have been analyzed, approximately 70% can be classed as ER-poor and 30% as ER-rich. During the past several years, ER assays have been carried out on approximately 700 mastectomy patients who had no indication of distant metastases. These women are being followed for recurrence of disseminated cancer and their response to endocrine therapy at that time. So far 5 of 6 patients with ER-rich primary tumors showed objective remission and one showed subjective remission to endocrine therapy at the time of recurrence (median recurrence 24 months) in contrast to only one remission in 15 patients with ER-poor tumors (median recurrence - 12 months). Antibodies to purified ER from calf uterus have been found to react with the estrogen receptor complex of human breast cancer cytosol; development of an ER assay in tumor slices by fluorescent antibody technique and in tumor cytosol by competitive RIA is in progress.

Significance for Cancer Research: (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $100,000 Site Visit Date: 10/23/73

CONTRACT RESEARCH SUMMARY

Title: Effect of Chemotherapy-Induced Endocrine Alterations on Stage II Breast Cancer

Principal Investigator:	Dr. Tapas K. DasGupta
Name/Address	University of Illinois Medical Center
Performing Organization:	Chicago, IL

Contract Number:
Starting Date: Expiration Date:

Goal: To determine whether the prolongation of the clinically disease free interval produced by adjuvant chemotherapy is a result 1) of direct anti-cancer action, 2) of indirect action via endocrine organ changes, or 3) of a combination of 1 and 2.

Approach: 1) In the clinical study, premenopausal women 50 years of age or younger with potentially operable breast cancer will be tentatively included for the purpose of obtaining preoperative hormone profiles. Blood sampling, when possible, will be done in both the follicular phase and the luteal phase. Radical or modified radical mastectomy will be performed. If one or more axillary nodes contain histologically proven metastases, the patient is eligible to enter the adjuvant chemotherapy program. Estrogen and progesterone receptor assays will be performed upon the breast cancer tissue. The study patients will receive 12 courses of cyclophosphomide, methotrexate, and fluorouracil (CMF). Blood hormone levels will be drawn during the first two weeks of each 28 day cycle of chemotherapy for three consecutive cycles and every three months thereafter during CMF administration. Correlations among receptor assay results, endocrine profiles, and tumor recurrences will be sought. 2) Animal in vivo studies will use adult female BUF/N rats and NMU induced mammary tumors. The effects of CMF on tumor, endocrine organs, and fecundity will be studied.

Progress: New contract

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D.
Program: Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: 0 Site Visit Date: 5/10/78

CONTRACT RESEARCH SUMMARY

Title: Endocrine Therapy Plus Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David T. Kiang
Name/Address University of Minnesota School of Medicine
Performing Organization: Minneapolis, MN

Contract Number: N01-CB-64000
Starting Date: 6/1/76 Expiration Date: 5/31/79

Goal: To study the effect on metastatic breast cancer of combining chemotherapy with estrogen therapy and to assess the value of estrogen receptor assays for treatment selection.

Approach: Patients eligible to enter this study program will be women who are showing the first evidence of metastatic breast cancer. Estrogen receptor (ER) assay results will determine the pair of treatment regimens to be randomly assigned after stratification according to disease-free interval and disease-dominant site. The patients with ER-negative cancer will receive (1) diethyl-stilbestrol (DES) plus cytoxan and 5-fluorouracil (CF) or (2) CF alone. Those with ER-positive cancers will receive either (1) DES and CF in combination or (2) DES alone followed by CF with evidence of DES treatment failure or of tumor progression after initial regression. The patient on whom ER assays cannot be obtained will have the same treatment assignments as those with ER positive tumors.

The incidence and duration of remission and survival data will be analyzed to determine (1) the effect of combining standard hormonal therapy and chemotherapy and (2) the value of ER assay results in predicting the clinical response to therapy.

Progress: Sixty-five patients with advanced breast cancer have entered the study. Forty-five of them were evaluated for response to randomized therapy. The overall response rate was 53%. The response rates in each subgroup are listed in the Table. The patient numbers are to small to reach any conclusion. Accrual of patients continues.

Response	ER (+)		ER (-)		ER (±) or ER (?)	
	DES	DES + CF	CF	DES + CF	DES	DES + CF
CR + PR	3/5	7/8	1/3	3/7	3/8	7/14
(Rate)	60%	87%	40%		37%	50%

Significance for Cancer Research: (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Marc Lippman, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $90,000 Site Visit Date: 2/11/76

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Dr. John M. Yuhas
Name/Address University of New Mexico School of Medicine
Performing Organization: Albuquerque, NM

Contract Number: N01-CB-74203
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The project will first explore the feasibility of growing six or more lines of rat mammary tumors *in vitro* as multicellular tumor spheroids (MTS). The characteristics of the *in vivo* tumors will be compared with those of the corresponding MTS. These characteristics will include transplantability into syngeneic rats or anergic mice, morphology, growth rate and growth fraction, cell cycle parameters, karyotype, and hormone receptors. If MTS are successfully developed, their responses to single drug doses will be studied and compared with *in vivo* responses. The *in vitro* MTS will be exposed to the test drugs in conditions which stimulate the *in vivo* conditions. When metabolic activation of drug is required, sera from drug-treated animals will be tested. The test drugs will be those which are commonly used in the treatment of human breast cancer.

Progress: A total of 10 mammary tumors (2 mouse, 2 rats, and 6 human) have been tested thus far for adaptation to growth as MTS. The rodent tumors adapted readily but only 2 of 6 human tumors grew as MTS. The 2 which did adapt were originally derived from solid growths (as were all of the rodent tumors), while the 4 which did not were isolated from pleural effusions. We are currently attempting to isolate both local recurrences and pleural effusions from individual patients to determine whether pleural effusions represent a selected variant subpopulation of tumor cells. An isotopic method has been developed that allows determination of both the growth rate and the growth fraction in the MTS. Through use of this method it has been demonstrated that the major factor responsible for variation in MTS growth rate is the growth fraction rather than the growth rate of individual cells. Our efforts to develop a medium for initial isolation of biopsy material have included defining of the requirements for established human mammary cancer lines. In at least one such line, an absolute requirement for insulin has been demonstrated.

Significance for Cancer Research: (NCP Objective 6 Approach 1,4)

Project Officer: Mary E. Sears, M.D., Richard Knazek, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $104,000 Site Visit Date: 1/18/77

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Name/Address Performing Organization:	Dr. Russell Hilf University of Rochester School of Medicine and Dentistry Rochester, NY

Contract Number: N01-CB-74204
Starting Date: 7/15/77 Expiration Date: 7/14/79

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: This project will perform assays for a battery of enzymes in primary and metastatic breast cancer specimens and build mathematical models of the biochemical profile. The enzymes include lactate dehydrogenase, pyruvate kinase, glucosephosphate isomerase, and isocitrate dehydrogenase. Hormone receptor assays and histological examinations will also be performed. Data concerning the hosts' biological and oncological histories will be acquired, evaluated, and stored. Analyses of the combined data will test the predictiveness of the mathematical model constructed from the biochemical profile.

Progress: Accession of new cases is occurring at the anticipated rate, and data collection and entry into computer file is proceeding. Forms for patient data at initial entry and followup were devised and are in use. More than 100 new samples have been received or analyzed with approximately 90% of the patients fitting our criteria for the study. Case review is continuing for patients whose tumors were assayed during the 7 years prior to initiation of this contract to update clinical course and therapy. At this time, two logistic regression models are being considered: one which uses LDH vs. PK x ICD x GPI and the second, which uses LDH vs. ICD/PGM. Results to date show a correct classification for response or no response of 84% for the former model based on 45 cases and 86% for the latter model, based on 37 cases. The data set is continually increased in number and is subjected to exploratory investigations, and instructions for entering patients submitting specimens and completing forms were given.

Significance for Cancer Research: (NCP Objective 6 Approach 1,4)

Project Officer: Mary E. Sears, M.D. and E. Brad Thompson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $88,200 Site Visit Date: 1/25/77

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Robert B. Livingston
Name/Address
Performing Organization: University of Texas Health Science Center
San Antonio, TX

Contract Number: N01-CB-74205
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: *In vitro* experiments will determine the effects of drugs on the thymidine labeling index of the rat mammary tumor 13762. The drugs will be added directly in pharmacologic concentrations or indirecly added via the serum of drug-treated animals. The *in vitro* effects will be compared with the *in vivo* effects on tumor size and on host survival. Cyclophosphamide, fluorouracil, methotrexate, vincristine, phenylalanine mustard, and adriamycin will be the drugs used in the test systems individually and in various combinations. If positive correlation between the *in vitro* and *in vivo* effects is found, additional experiments will test the predictivesness of the *in vitro* effects for the development of metastases and with the survival of animals from which the advanced local tumors have been excised.

Progress: Cyclophsasphamide and vincristine have been tested in this system as individual drugs. Each has *in vivo* antitumor effect. However, the sensitivity of the tumor to cyclophasphamide has been variable in our experience. When tumor regression meeting the usual clinical criteria for response was observed, corresponding *in vitro* depression of labeling index in drug-containing plasma, relative to control plasma, was observed for cyclophosphamide. Problems in the test system that require further work include: 1) non-drug-specific depression of labeling index secondary to the use of plasma as an incubating medium, which makes drug effects difficult to evaluate; and 2) tendency of the tumor cells to aggregate into large clumps *in vitro*. Approaches have been formulated to attempt to solve these problems.

Significance for Cancer Research: (NCP Objective 6 Approach 1,4)

Project Officer: Mary E. Sears, M.D., and Richard Knazek, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $54,900 Site Visit Date: 1/17/77

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator: Name/Address	Dr. Federico Welsch Worcester Foundation for Experimental Biology
Performing Organization:	Shrewsbury, MA

Contract Number: N01-CB-43867
Starting Date: 10/16/78 Expiration Date: 10/15/78

Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: This is a type I contract in that the contractor carries out determintations of estrophilic receptor protein (ER) levels on 650 breast cancer samples per year. These tissues are submitted by collaborating clinicians whose protocols have been approved by the project officers. The ER assays are performed by both the sucrose gradient and the charcola methods, and methodology is under constant monitoring. Histological examination by St. Vincent's Hospital is being carried out on each tissue submitted for ER assay. The contractor also performs steroid sulfurylation determinations on tumor samples of sufficinet size. Progesterone receptor assays have recently been added.

Progress: The contractor has analyzed more than 1700 specimens received from five institutions. Forty-five percent of the tumors were found to bind >10 femtomoles of estradiol per mg protein, 14% bound from 3 to 10 femtomoles/mg and 41% bound <3 femtomoles/mg. Forty tumors have been analyzed for progesterone receptor and 495 determinations made of sulfurylase activity by the Brooks method and 187 by the Dao method. The data are currently being correlated with the clinical onset and course of the breast cancers.

Significance for Cancer Research: (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 78 Funds: $125,300 Site Visit Date: 6/7/73

ANNUAL REPORT SUMMARY
CANCER DIAGNOSIS CONTRACT PROGRAM
October 1, 1977 to September 30, 1978

The Diagnosis Branch of the Division of Cancer Biology and Diagnosis has the major responsibility for the Diagnosis Program of the National Cancer Institute. This program has operated almost entirely by the contract mechanism with the exception of several CREGs. With the recent administrative change, diagnostic grants will now be administered by this Branch. We will have an opportunity to choose more adequately the most desirable mechanism for administering research. The program has been defined and the contracts awarded and monitored with the expert advice of five individual Advisory Committees, one for each of the specific areas of involvement: General Cancer Diagnosis, Diagnostic Radiology, Cytology Automation, Breast Cancer, and Immunodiagnosis. The programs for the last two are presented in this Annual Report in separate sections; those for the first three are presented below.

GENERAL CANCER DIAGNOSIS STUDIES

Lung Cancer All three institutions (Mayo Clinic, Johns Hopkins University Medical School, and Memorial Hospital-Strang Clinic) have entered their full quotas of screenees including controls. All three institutions continue to identify early lesions, both by cytology and by radiology, in an effort to permit lesion removal and cure by a surgical procedure less extensive than pneumonectomy. The University of Cincinnati Statistical Center continues to coordinate accumulation of data from the three cooperating clinical projects. Mortality review continues to be a major part of the study. A Manual of Procedures, compiled early in 1975 and for which there have been considerable requests, has been reviewed, brought up to date, and will be ready for distribution soon.

Pancreas Cancer The three cooperating institutions, Mayo Foundation, University of Chicago, and Memorial Hospital, have finished their patient input into this study, which attempted to define diagnostic methods with a high probability of finding pancreatic cancer at surgical exploration. In the process, the institutions have collected the largest group of possible pancreatic cancer, which have been examined by almost all known pancreatic cancer detection devices. Proceeding with surgical exploration, the three institutions have thus been able to establish many negative findings, as well as the positive finding that ultrasound (using gray scale), CAT scanning, and retrograde pancreaticoduodenography together will come close to identifying all pancreatic cancers (small pancreatic cancers were not among those explored or seen). Final reporting based on the data collected at the University of Cincinnati Statistical Center will take place within the next year.

Colonic Cancer The American College of Radiology continues to investigate the relative values of the twelve methods of bowel preparation prior to barium enema or colonoscopy. The University of Louisville continues to investigate the development of an immunologic rapid method for the detection of human blood in stool. The University of Minnesota has recruited its full quota of screenees in its investigation of a screening technqiue using a test for blood in the stool as a means of detecting early bowel cancer, thereby possibly prolonging lives. This study includes a control group and two study groups, one screened annually and one screened biennially.

Bioch mical Markers Roswell Park Memorial Institute has completed its development of a counter-immunoelectrophoretic method for the detection of specific prostatic acid phosphatase in prostatic cancer. The method is now being tested in a field study.

Hormonal Markers Harbor General Hospital has now completed its investigation of hormonal markers in lung cancer, colonic cancer, and breast cancer; data analysis and final reporting of the results will be completed soon.

Multisite Screening studies at the University of Tennessee have now been completed.

Systems to Improve X-ray Imaging by removing scatter have been developed and tested at the University of Alabama and the University of Wisconsin.

Periodic Screening for Medullary Thyroid Carcinoma is being done with relatives of patients at Duke University to investigate possibilities of altering mortality from this disease.

Immunodiagnostic Methods of Detection for Ovarian Cancer are being developed and tested at Tufts University College of Medicine and at Roswell Park Memorial Institute.

The Development of a 5'-Nucleotide Phosphodiesterase Serum Enzyme Test for human liver cancer is being done at the University of Pennsylvania.

The University of Wisconsin School of Medicine is defining more clearly the possible diagnostic significance of the presence of special isozyme of alkaline phosphatase in the sera of human cancer patients.

Two new contracts in response to RFPs are being negotiated and will probably be funded before the end of the fiscal year. One contract will address the subject of establishing the feasibility of obtaining endometrial samples by three different methodologies to detect endometrial cancer earlier; the second will be directed toward improving imaging modalities in the diagnosis of pancreatic cancer.

DIAGNOSTIC RADIOLOGY

In this area of research, improvement of x-ray imaging has been a matter of major concern and one to which the Diagnostic Radiology Committee has devoted much time. For early cancer detection and for screening programs involving periodic recurrent x-rays of individuals, it is extremely important to achieve improved resolution with substantially lower levels of radiation.

Five institutions, Massachusetts General Hospital, Columbia Presbyterian Medical Center, the Mayo Clinic, George Washington University Medical School, and the Cornell University Medical School, have projects to perform clinical evaluation of equipment for computer-assisted tomography (CAT) of the brain, a noninvasive technique believed to be of great promise in the field of neuroradiologic diagnoses. This technique is expected to complement, and in some instances, replace present techniques used in diagnosis of intracranial lesions. Enrollment of patients was completed during the summer of 1977; follow-up studies will continue for two subsequent years. Extensive data have been obtained in this collaborative study; a number of reports are being prepared for publication based on an analysis of these data.

A project to investigate use of proton beams and heavy ion beams in tissue density measurement continues at the DOE-Lawrence Berkeley Laboratory. Final evaluation of possible clinical usefulness of the imaging method cannot yet be made.

In addition to the above six projects, all of which started in June 1974, seven additional projects started in June 1975. All seven were concerned with various aspects of development of new cancer diagnostic capabilities using CAT. One contract, with Mayo Foundation, concerned accumulation of a transaxial x-ray projection data base for use in testing algorithms; another, with Research Foundation of SUNY, supported development of algorithms for computerized transaxial x-ray reconstruction. Both of those contracts, now completed, were most productive. The Mayo contract, which terminated in June 1977, has produced an extensive projection data bank which will be made available by NIH to other interested groups. The SUNY contract, which was completed in January 1978, has provided new useful algorithms for x-ray reconstruction. Three contracts, with DOE-Lawrence Berkeley Laboratory, University of Pennsylvania, and Massachusetts General Hospital, involved development of algorithms for computerized transaxial nuclide reconstructions. The last two were completed in June 1977; final reports will be available soon; the one with DOE-LBL will terminate in June 1979. All three are producing a variety of new and useful algorithms for nuclide reconstructions; these will be made available to interested groups. Two contracts, with American Science and Engineering and Presbyterian Hospital (NY), have supported development and evaluation of a CAT body scanner. The scanner was completed and installed by AS&E at Presbyterian Hospital in May 1977. After one year of clinical evaluation, which indicated an extremely good imaging capability for the scanner, both contracts were terminated in May 1978.

During June 1976, four new contracts related to Diagnostic Radiology were started. One, with Bolt Beranek and Newman, produced a standard protocol for use in evaluating imaging techniques used for cancer diagnosis. The tentative standard protocol was developed and pilot-tested during the summer and fall of 1977. Following some modifications, the standard protocol is being used to evaluate results accumulated from two other collaborative studies in the Diagnosis Program. That work will be completed in December 1978. Two other contracts, with the University of Kentucky and the University of Illinois, are supporting studies to produce tumor-specific gamma-labeled immunoglobulins to allow tumor detection by external means. The fourth contract, with the University of Arizona, supports development of radioisotopically labeled markers (which will accumulate in small cancer lesions in mucosal surfaces) and the development of suitable detectors to locate such accumulations on endoscopically approachable surfaces.

During the summer of 1977, five new contracts were started. Two of those contracts, with General Electric Company and Xerox Corporation, are for the development of large area solid state image receptors for x-ray images. One contract, with SRI, is for the development of ultrasonic probes to be inserted through endoscopes for improved cancer diagnosis in or near endoscopically approachable areas of the body. The remaining two contracts, with Northwestern University and Lahey Clinic Foundation, seek development of new techniques for improved passage of colonoscopes to the cecum to promote improved early diagnosis of bowel cancer.

New contracts to be negotiated during the summer of 1978 involve studies in: (1) methodology using contrast agents to improve detection of small liver metastases with x-ray computerized tomography; (2) development of contrast agents for use in clinical ultrasonic diagnosis; and (3) reconstruction algorithms for dose reduction in x-ray computerized tomography.

CYTOLOGY AUTOMATION CONTRACT PROGRAM

The Committee on Cytology Automation was established in July 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute, on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation in operational terms for National Cancer Institute's contractual support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Committee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

At the present time, work in several very important areas of research has been accomplished. In area 1, specimen collection, Dr. Marluce Bibbo and collaborators at the University of Chicago determined in an extensive study that the number of single abnormal cells present in gynecologic specimens is adequate for screening purposes in essentially all cases of pre-malignant and malignant lesions. Thus, only relatively gentle disaggregation procedures are necessary in preparing clinical specimens for automated analysis.

In area 2, specimen preparation, several contractors have completed a thorough examination of mechanical and enzymatic dispersal procedures to increase the yield of single intact cells. The results of this work indicate that relatively simple mechanical dispersal techniques, such as pressure-controlled syringing and ultrasonic treatment, produce adequate numbers of morphologically and cytochemically intact cells for instrumental analysis.

In areas 3, 4, and 6, development and evaluation of integrated screening systems, several automated analytic systems have been identified as promising potential designs for automated clinical prescreening systems. Evaluation and development of these systems is proceeding to: (1) ensure that they reflect a sound biologic basis for identification of pre-malignant and malignant squamous cell lesions of the gynecologic tract; and (2) evaluate their performance characteristics, especially preliminary analysis of sensitivity and specificity.

Work is continuing on the identification of quantitative descriptors of premalignant and malignant lesions, especially cytochemical and immunological markers which will increase the potential sensitivity and specificity of currently employed sensor systems in screening clinical material. In addition, research is in progress to test a module sorter to separate cells based on individual cell parametric measurements, assuring a correlation of individual separated cells with specific quantitative measurements in flow systems. The module will be interfaced to a multiparameter flow sorter. Research is developing and testing single cell classification algorithms employing the current state of the art in digital image processing, scene segmentation, and image acquisition hardware. An integral system and algorithms are being evaluated to determine the classification performance for screening squamous epithelial cells. Work has started in the comparison of instrumental or system classification and microscopic "true classification" using standard morphologic criteria. The data will have a direct bearing on the analysis of the performance of any automated systems.

CONTRACT RESEARCH SUMMARY

Title: Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator:	Dr. Arthur J. Present
Name/Address	American College of Radiology
Performing Organization:	Chicago, IL

Contract Number: NO1-CB-43974
Starting Date: 6/15/74 Expiration Date: 9/29/78

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time, excessive dietary restrictions, or excessive patient discomfort or inconvenience.

Approach: Under administrative supervision of the American College of Radiology, the comparative efficiency of 12 selected colon cleansing regimens are being studied by six institutions. A total of 3,600 patients are being examined; all regimens will be employed by each institution on a randomized basis. Standard forms for collection of pertinent clinical information and for evaluation of efficiency of each regimen have been prepared. A panel of readers will independently examine X-ray film taken on the patients and evaluate the relative clarity of the bowel. A method for rerandomized distribution and evaluation has been requested per the last site visit. This will continue to encompass methods for cross evaluation. Statistical analyses will be performed by the Department of Biomathematics at M.D. Anderson Hospital.

Progress: The project is continuing with participation of six centers and several other readers. The protocols have been met in each instance, and now a re-examination of films with a new technique of randomization is being undertaken. Whereas each technique was evaluated by several readers before, now each reader will compare the 12 specified protocols in one set of unidentified but numbered films. It is felt that this will give us a further insight into the effectiveness of each method and continue to give us an assessment of the individual reader. The number of readers has been increased in this improved method of randomization of film reading.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officers: Louis P. Greenberg and R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 4/16/76

CONTRACT RESEARCH SUMMARY

Title: Screening for Medullary Carcinoma of the Thyroid Using Calcitonin Radioimmunoassay

Principal Investigator:	Dr. Samuel A. Wells
Name/Address	Duke University
Performing Organization:	Durham, NC

Contract Number: N01-CB-63994
Starting Date: 6/15/76 Expiration Date: 6/14/81

Goal: The goal of this project is to establish a periodic screening program of relatives of patients with medullary carcinoma of the thyroid gland (MCT).

Approach: A cohort of patients in which MCT is proven histologically will be established. The cohort will be sufficiently large to offer a statistically significant number of family members. Those family members will be screened for serum calcitonin levels by radioimmunoassay methods in order to find MCT during its early stage of development. A long-term followup program will be conducted on these families to determine the natural history of the disease and effectiveness of therapy.

Progress: Since inception of the contract, 389 patients have been screened, and the total number of cases of multiple endocrine neoplasia type II now stands at 90. Additionally, we have four patients with multiple endocrine neoplasia type IIB. Members of seven kindreds are being evaluated on a semi-annual or annual basis. Since we have begun to use the combined stimulation test of calcium gluconate (2 mg/kg/1 min.) and pentagastrin (0.5 mcg/kg/5 sec.) we have diagnosed a large number of patients with C-cell hyperplasia. This combination test has proven superior to either calcium gluconate or pentagastrin given alone. Furthermore, using selective thyroid venous catheterization with provocative stimulation has allowed us to diagnose a large of number of patients with microscopic disease.

Of four patients treated with chemotherapy (Adriamycin, Cis-platinum, and Streptozotocin), only Adriamycin seems to be of benefit.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $98,733

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator:	Dr. William D. Odell
Name/Address	Harbor General Hospital
Performing Organization:	Torrance, CA

Contract Number: N01-CB-43903
Starting Date: 6/1/74 Expiration Date: 5/31/78

Goal: To develop hormone markers that might indicate the presence of small (early), treatable cancer.

Approach: (1) To measure ADH, vasotocin, oxytocin, α and β MSH, ACTH, α and β TSH, and α and β HCG to search for tumor elaboration of these peptides among cancer patients; (2) to develop new techniques for separating and identifying "prohormone" forms of ACTH, gastrin, and pancreozymin-cholecystokinin (PZ-CCK); (3) to search for the existence of such big forms of ADH, oxytocin, and β MSH, luteinizing hormone, and α and β glycopeptides, (4) to develop peptide assays for fetal hormones related to vasopressin; and (5) to identify other nonhormonal peptides which might be used as cancer markers. The contractor hypothesized that "ectopic peptide elaboration is a universal concomitant of neoplasia." This hypothesis is based on a review of several hormonal syndromes produced by nonendocrine cancers and the fact that, where known, they are produced by peptides. Production of nonbiologically active peptides is even more common than production of active peptides.

Progress: Using radioimmunoassay ACTH was identified in all (N =∽ 80) extracts of cancer of the lung, colon, pancreas, and stomach. This material did not react in the radioreceptor assay and had a larger molecular weight than standard ACTH. It bound to Concanavalin-A and was believed to be pro-ACTH. Samples from over 800 patients with various diseases or diagnostic possibilities were studied, and clinical correlations were established. In prospective studies of chest disease, it was established that blood pro-ACTH was elevated in 72%, beta-lipoprotein was elevated in 62%, hCG was elevated in 7%, vasopressin-vasotocin was elevated in 41%, and free alpha chain was elevated in 9% of patients with cancer. Patients with benign disease did not have elevated values of these peptides. In extracts of all normal human tissues tested, hCG was identified. It was shown that this normal tissue hCG did not bind to Concanavalin and this was carbohydrate-free or had markedly different carbohydrate content from placental hCG. Extracts of all carcinomas studied also contained hCG, and the amount was similar to that in normal tissues in 77% of cancers. In 23% tumor extracts contained more hCG than was found in normal tissues. It was also demonstrated that those cancers associated with detectable blood hCG were associated with hCG showing increase Concanavalin binding, i.e., increased amounts of carbohydrate-rich hCG. We concluded that all normal tissues and all carcinomas elaborate hCG and that the variable in hCG detection is that some cancers glycosylate the peptide and/or overproduce the peptide.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 3/14/77

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: Name/Address Performing Organization:	Dr. John K. Frost Johns Hopkins University Baltimore, MD

Contract Number: NO1-CN-45037
Starting Date: 9/1/73 Expiration Date: 7/15/79

Goal: To determine the effect of the early detection of lung cancer by sputum cytology, followed by surgical removal of the tumor on long-term survival in the disease (Phase II of Hopkins Study).

Approach: The study population consists of males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment from individuals responding to advertisement, it was planned to accumulate over several years two groups of over 5,000 each. The test group would receive sputum cytologic examination and chest X-ray every four months; the control group will have an annual chest X-ray. Subjects suspicious or positive for cancer would be carefully worked up, to include fiberoptic bronchoscopy; cancers would be removed surgically. All subjects will be followed for at least 5 years to determine actuarial survival. (See also NO1-CB-92172.)

Progress: A total of 10,362 people have been enrolled in this project, of which 4,764 were randomized into an "X" Control Group and 4,804 into a "C" Study Group. To date, 280 have died, while 514 have withdrawn from the study. Thus, 9,568 remain as active participants; 4,764 in the "X" Group and 4,804 in the "C" Group. At initial screening, 71 cancers were detected, for a detected prevalence of 6.9 per 1,000. Between the initial negative screening and the first annual examination a total of 83 cancers appeared (prevalence: 8.1 per 1,000). There has been a sensitivity of 87.2% and a specificity of 99.5%. In the detected cancers of the "C" Study Group, 76% were detected by X-ray and 47% by cytology. An X-ray "CAN" yielded cancer 51% of the time; a cytology "CANCER" yielded cancer 100% of the time. Fifty-one percent of these prevalence cases and 55% of the incidence cases were detected in the earliest stages (0 and 1). The advanced stages II and III show a survivorship of 15% at 36 months. All prevalence cases showed a 48% survivorship at 36 months.

Significance for Cancer Research: (NCP Objective 5 Approach 4)
Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $1,095,000

CONTRACT RESEARCH SUMMARY

Title: Lung Project for Detection and Localization of Early Lung Cancer

Principal Investigator:	Dr. Robert S. Fontana
Name/Address	Mayo Foundation
Performing Organization:	Rochester, MN

Contract Number: N01-CB-53886
Starting Date: 9/27/74 Expiration Date: 9/26/78

Goal: To test new methods of diagnosis of early lung cancer and to assess survival of patients with lung cancer detected by these methods.

Approach: A study population of 9,313 subjects was obtained from interviews of 11,000 non-volunteers. Subjects were Mayo Clinic outpatients who were men, 45 years old or more, smoking one pack of cigarettes or more daily. None had a history or suspicion of respiratory cancer on entry into the Clinic; all had a life expectancy of at least 5 years, and all were judged capable of tolerating pulmonary resection. The 9,313 study subjects were first screened by means of chest X-rays, 3-day sputum cytology tests and lung health questionnaires. This screening yielded 86 unsuspected ("prevalence") lung cancers, 16 detected by cytology alone, 53 by X-ray alone, and 17 by both tests. Radiographically "occult" cancers were localized fiberbronchoscopically. Subjects with a "negative" initial screen were randomized into a close surveillance group, rescreened every 4 months, and a standard surveillance group ("control") advised to undergo rescreening once a year. Potential lung cancer "risk-factors," including aryl hydrocarbon hydroxylase (AHH) are also being evaluated, as are hematoporphyrin derivative (HpD) and cryotherapy as techniques for "mapping" and treating carcinoma-in-situ.

Progress: As of April 1, 1978, both the close surveillance group and the "control" group had been observed approximately 15,000 man-years, and 117 new ("incidence") cases of lung cancer had been detected. In the close surveillance group there were 71 new lung cancers (11 of which were detected cytologically) and 26 lung cancer deaths. Survivorship in this group is encouraging, but longer observation is needed. In the control group there were 46 new lung cancers and 26 lung cancer deaths, with a tendency toward more traditional lung cancer survivorship. The three-year survival rate of the "prevalence" cases was 44%.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $998,000

CONTRACT RESEARCH SUMMARY

Title: Usefulness of Carcinoembryonic Antigen in the Diagnosis of Bowel Cancer

Principal Investigator:	Dr. Vay Liang W. Go
Name/Address	Mayo Foundation
Performing Organization:	Rochester, MN

Contract Number: NO1-CB-23854
Starting Date: 6/15/72 Expiration Date: 6/14/79

Goal: (a) Evaluate the CEA test in diagnosis of large bowel carcinoma; (b) continue evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy, and immunotherapy of advanced gastrointestinal cancer; (c) measure CEA levels in blood and other specimens stored at NCI-Mayo Clinic Tumor Plasma Bank (CB-23879), Mayo Lung Cancer Project (NCI-CB-22000) and other contracts with the Immunodiagnosis Program of the Division of Cancer Biology and Diagnosis; (d) immunological quantification and chemical characterization of CEA-like material in secretions of the gastrointestinal tract; (e) continue to collect and provide colonic malignant tissue for different investigators in projects dealing with carcinoembryonic antigens.

Approach and Progress: (a) Results of serum CEA evaluation of 6,700 patients were previously reported. Follow-up survival data of the patients who had colorectal cancer at the time of initial CEA determination has also now been obtained. Among the colorectal cancer patients with localized or with distal metastases involving the liver patients with initial serum CEA levels less than 5 ng/ml have a much better survival rate than patients with CEAs greater than 5 ng/ml. (b) Serial determinations of serum CEA have been done on 612 patients with far advanced gastrointestinal malignancies who are currently being treated under various approved NCI protocols receiving cancer therapy, including 236 patients with colorectal cancer who were operated on with curative intent. Changes in serum CEA levels have been correlated with patient course and effects of cancer therapy. Manuscripts have been submitted and accepted for publication in JAMA and Annals of Internal Medicine. (c) We determined serum or plasma CEA levels in 9,467 specimens for the NCI-Mayo Clinic Tumor PLasma Bank in 1977. Collaborative studies with Dr. Hersh (CB-43887) showed that CEA-S measurement in prospective studies did not show comparative advantages over CEA determination. (d) CEA-like material from normal human colon washings had similar amino acid and carbohydrate composition to tumor CEA.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 2/9/77

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Dr. Vay Liang W. Go
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: N01-CB-43895
Starting Date: 6/15/74 Expiration Date: 2/28/78

Goal: (a) To determine values of newer diagnostic tests in the detection of pancreatic cancer; (b) to determine whether surgical intervention of a localized pancreatic cancer significantly improves survival rate; and (c) to develop new procedures for pancreatic cancer diagnosis.

Approach: Three cooperating institutions will study, by a common protocol, patients who are suspicious of pancreatic cancer (PC) and warrant exploratory laparatomy. Tests to be done prior to surgery: CEA, alpha-fetoprotein and immunoglobulins, duodenal aspiration and study of pancreatic enzymes (PFT) and biliary secretion, ultrasonic abdominal study (U), fiberoptic duodenoscopy with retrograde pancreatocholangiography (RPGM), pancreatic scan with ^{75}Selenomethionine (S), selective celiac and/or superior mesenteric angiogram (A), and thermogram (T). At operation, portal blood will be obtained for additional studies. If pancreatic cancer is found at surgery, the patients will be evaluated at 3-month intervals for 3 years. If no cancer is found, patients will be evaluated at 6-month intervals for 3 years.

Progress: This study was completed on February 28, 1978. Two hundred seventy-two patients were entered into the study of which 246 had laparotomies, and 108 patients were found to have pancreatic cancer. Analysis of the prospective comparison of the various current diagnostic tests has now been completed and has resulted in various publications: N Engl J Med 297:737-742, 1977; Gastroenterology 73:457-461, 1977; and Radiology 124:731-737, 1977. The results of the various blood tests have not been analyzed and the manuscripts are in various states of preparation for submission for publication.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 3/21/77

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer -- Early Detection, Localization and Therapy

Principal Investigator: Name/Address Performing Organization:	Dr. Myron R. Melamed Memorial Hospital for Cancer and Allied Diseases New York, NY

Contract Number: N01-CN-45007
Starting Date: 9/1/73 Expiration Date: 8/31/78

Goal: To evaluate sputum cytopathology as a supplement to annual chest X-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to X-ray positive); in general, to evaluate the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed.for at least an additional 5-year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: The early lung cancer detection program in New York is now in its fifth year. A total of 10,006 cigarette smoking men over 45 years of age have volunteered and enrollment into the study is complete. Of the men, 4,948 were randomly allocated into "group A" who have annual PA and lateral chest X-rays with four-monthly sputum cytology examinations, and 5,058 were randomly allocated into "group B" having annual PA and lateral chest X-rays without sputum cytology. A total of 103 men with lung cancer have been identified so far; 50 were found on initial examination (Prevalence = 5.0/1000), and 53 have been found subsequently. Thirty-four of the 103 cases were very early when discovered (Stage 0 or I). In group B there were 14 cases in Stage I discovered by X-ray and 9 cases in Stage 0 or I discovered by cytology. All have been treated surgically. Thirty-one are living and are free of disease; 2 died of other causes without residual or recurrent lung cancer, and only 1 of the 34 men has died of his lung cancer. We are continuing to follow all of the men in the program to determine the incidence of new lung cancers according to stage and tumor type, the efficiency of the detection program, and the effect of the program on the death rate from lung cancer.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $875,246

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Malaya Bhattacharya
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, NY

Contract Number: N01-CB-64013
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: The goal of this project is the development of sensitive immunoassay procedures for early diagnosis of ovarian cancers and serial monitoring of patients undergoing therapy for ovarian cancers.

Approach: The investigation will be conducted on the following topics: (1) preparation of antisera (anticystadenocarcinoma sera); (2) sources of ovarian cystadenocarcinoma-associated antigens (OCAA); (3) purification of OCAA by conventional methods, immunoadsorbent columns, affinity columns of plant lectins; (4) determination of purity of OCAA; (5) characterization of OCAA; (6) determination of the frequency of occurrence of OCAA in other cancer and noncancer tissues; (7) development of a radioimmunoassay for detection of OCAA; (8) patient selection, availability, and followup; (9) characterization of immune response to ovarian cancers with emphasis on cystadenocarcinoma; and (10) problems and limitations of the proposed projects.

Progress: Evidence has been found for the presence of at least three different tumor-associated antigens or antigenic determinants in human cystadenocarcinomas of the ovary. Solid ovarian tumors, tumor cells in ascites fluids and cyst fluids from ovarian cancer patients were used as sources for these antiigens. After proper adsorptions the antisera produced were used to perform the assays for tumor-associated antigenic reactivity. For quantitative isolation of these antigens repeated extractions separately or in combinations with 3M KCl, 6M urea and normal saline were performed; 3 time extraction with saline gave the best results. Ammonium sulfate fractionation of the saline extracts gave about six- to ten-fold purification. Further fractionation by gel filtration and ion-exchange chromatography on QAE-Sephadex has yielded 120-fold purification of the antigens. OCAA yielded 2 peaks (PK 1 and 2) on QAE-Sephadex which were iodinated by Bolton-Hunter reagent and checked for purity. PK 1 moved as a single peak on polyarcylamide gels and on isoelectric focussing. However, it was resolved into 3 peaks on Con A-Sepharose and 2 peaks on hydroxylapatite. Antisera against all these different molecular species are being raised in rabbits after isolation from tumor and radioimmunoassay for PK 1 is being developed. We are also trying to purify PK 2 of OCAA. By negative affinity chromatography, the second ovarian tumor antigen was further purified. On iodination, it still showed at least 2 major peaks, indicating the need for further purification.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $50,384 Site Visit Date: 12/14/76

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Leo Stolbach
Name/Address Tufts University School of Medicine
Performing Organization: Boston, MA

Contract Number: N01-CB-64028
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: The goal of this project is to develop a screening test for the early diagnosis of ovarian carcinoma in asymptomatic women.

Approach: Malignant effusion of patients with ovarian carcinoma will be processed in order to (1) isolate and purify tumor-specific antibodies and/or antibody-antigen complexes from effusion fluids; (2) dissociate the antibody-antigen complexes and separate the tumor-associated antigens and antibodies making up these complexes; (3) determine the immune specificity of antibodies isolated from the effusion fluids; (4) detect tumor-associated antigen in the effusion fluid and tumor extracts; (5) utilize the tumor-specific antibodies to prepare an immunoadsorbent for the purification of the antigen; and antibodies to prepare an immunoadsorbent for the purification of the antigen; and (6) develop a diagnostic assay for detecting circulating antibody, antigen and antigen-antibody complexes in patients with various stages of ovarian malignancy.

Progress: Antibodies have been isolated from effusion fluids of 47 patients with cancer of the ovary, and 9 of these patients have been demonstrated to have specificity for ovarian cancer cells by indirect immunofluorescence (IF). An antiserum was prepared by injection of rabbits with immune complexes isolated from the effusion fluids of ovarian cancer patients. After extensive absorptions, the antiserum was found to give IF titers of 1024 to 256 against ovarian cancer cells and 8 against normal ovarian and control cancer cells. The following immunoabsorbents have been prepared: 1) the extensively absorbed rabbit antiserum; 2) a pool of relatively specific patient antibodies; 3) a pool of patient antibodies demonstrated to be highly specific for ovarian cancer cells; and 4) antibodies from a patient with a very high titer. Normal ovary, ovarian tumors, and control tumor tissue extracts have been prepared and placed on the immunoadsorbent columns. The eluates from the different extracts and immunoadsorbents are being characterized by analytical techniques. Material(s) with similar characteristics found in the column eluates of ovarian cancer patients and not detected in other types of cancer or normal specimens will be considered candidate "antigens." These "antigen materials" will be used as immunogens to prepare a rabbit antiserum of necessary specificity to develop a radioimmunoassay for the early diagnosis of ovarian cancer in asymptomatic women.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $111,931 Site Visit Date: 12/13/76

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: Name/Address Performing Organization:	Dr. C. Ralph Buncher University of Cincinnati Medical Center Cincinnati, OH

Contract Number: N01-CB-43868
Starting Date: 6/21/74 Expiration Date: 5/31/79

Goal: To collect and analyze data from the three collaborating clinical groups in the lung cancer screening program directed toward the detection of early lung cancer in high risk patients to see if this screening program will reduce mortality and morbidity.

Approach: Procedures have been established and agreement has been reached concerning the common data base for this study. The data are being monitored by the Statistical Center, entered into the computer, and analyzed. Reports are being made quarterly. The Statistical Center will search the data for significant statistical findings.

Progress: In the Cooperative Early Lung Cancer Group program, the Cincinnati Central Statistical Group (CSG) has established a common data base, made suggestions for uniform data recording by the clinical centers, and reported results to the NCI and participating clinical centers. The CSG receives study data from each of the clinical centers quarterly and forms a master patient file from which tables, reports, and analyses are derived towards answering the questions of the study, i.e., whether efforts directed toward earlier diagnosis have successfully led to improvement in mortality from lung cancer.

Tables showing the combined results and status at each of the clinics are produced routinely. They have proven of value to the NCI and other participants as a means of monitoring the progress and results of the study. Patient status indicator codes and information on follow-up have been added to the common data base, and computer programs for editing submitted information have been developed. In the past year, programs to calculate person-years of risk have been written, and mortality records have been computerized.

This contract has included similar work in a three-center study of pancreatic cancer and a five-center study of brain cancer; final reports for those studies are being prepared.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection of Small Amounts (over 7-10 ml/24 Hours) of Human Blood in Human Feces

Principal Investigator: Dr. Curtis L. Songster
Name/Address University of Louisville Foundation, Inc.
Performing Organization: Louisville, KY

Contract Number: N01-CB-43968
Starting Date: 6/30/74 Expiration Date: 9/30/78

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 ml in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing antigens specific for the globin portion (alpha and beta chains) of hemoglobin. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry, and fish diets and be truly negative by the proposed test.

Progress: High titer antisera have been raised against alpha and beta chains of human hemoglobin. Cow, pig, sheep, chicken, horse, and fish hemoglobins lack cross-reaction. Cross-reactions with human hemoglobins are complete for A/A, A/S, A/C, S/S, S/C, and monkey. Rabbit hemoglobin is weakly cross-reactive, but can be absorbed out and purified by DEA exchange. Radial immunodiffusion techniques using anti-alpha-chain and anti-hemoglobin appear capable of specifically detecting human blood at levels of 2 mg% in purified A/A hemoglobin hemolysates. Fabricated fecal bleeds representing 3 to 100 milliliters of whole blood/24 hours, can be routinely detected in stool specimens with no false positives. In contrast hemoccult has only a 5% detection rate with a 2% false positive error. Methods to increase sensitivity are being evaluated. Multiple specimen testing of preoperative patients with documented lesions is being studied to determine amount and frequency of bleeding, and currently numbers over 150 cases. Analysis, collation, and interpretation of over 6,000 stool samples is in the concluding phase for final data reduction.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 4/13/76

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool to Detect Early Cancer of Bowel

Principal Investigator: Name/Address Performing Organization:	Dr. Victor A. Gilbertsen University of Minnesota Minneapolis, MN

Contract Number: N01-CB-53862
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: The goal of this project is to demonstrate a significant reduction in mortality due to colorectal cancer between the annually screened and the control group by employing the Hemoccult (R) form of the guaiac test for stool blood in combination with a diagnostic protocol for the source of bleeding.

Approach: Forty-five thousand participants between the ages of 50 and 80 years residing in the state of Minnesota are randomly allocated to three groups on the basis of age, sex, and geographic location in the state. Slides are completed after the observance of a meat-free diet with suggested high fiber content. One of the groups completes the slides once per year, another every other year, and a third group serves as the control. Participants submitting samples positive for blood receive a diagnostic examination (including a colonoscopy) to determine the cause of bleeding. All diagnostic procedures are conducted at the University of Minnesota Medical Center. All data including pathology reports on biopsy material and surgical specimens are computerized for easy retrieval. Extensive follow-up procedures are designed to retain participation of all subjects throughout the five years of testing and an additional five years of followup. Extensive dietary and health history data are collected from each subject via questionnaire.

Progress: Response rate after the first year of testing is 85% for test slides and 90+% for questionnaires (control group). Tested groups receive questionnaires at time of notification of negative slide results or at the time of diagnostic procedure should tests be positive. One hundred percent of the 45,000 participants was recruited as of November 1977.

Portions of the initial cohort (first tested February 1976) have completed the second year of testing. Rehydration of slides prior to development was started in June 1977. A substantial increase in the number of positive slide readings seems to be correlated with this change in procedure. The implications for the study are being evaluated.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnosis Research Advisory Group
FY 78 Funds: $609,000 Site Visit Date: 4/20/76

CONTRACT RESEARCH SUMMARY

Title: Development of Serum Isozyme Test for Human Cancer Patients

Principal Investigator: Dr. K. C. Tsou
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, PA

Contract Number: N01-CB-64029
Starting Date: 6/30/76 Expiration Date: 4/30/79

Goal: To improve on the early diagnosis of cancer of the liver and further examine the possibilities of preventing the spread of primary cancers to other body sites with concentration on liver tissue.

Approach: The project involves: (1) further technical improvement of the 5'-nucleotide phosphodiesterase isozyme method by gel electrophoresis as a diagnostic tool; (2) further development of correlation data (biopsy, scan) and 5'-nucleotide phosphodiesterase-V for primary hepatoma; (3) further definition of possible relationships of the isozyme band V of this enzyme to liver metastasis; (4) investigation of possible relationships between this isozyme and other known liver markers related to hepatic cancer, especially α-fetoprotein and hepatitis B surface antigen (Australia antigen); (5) improvement of sensitivity of the test through development of an alternate assay method, including immunoassay if an antienzyme antibody can be prepared with reasonable ease; (6) correlation of clinical information and other liver function tests with the results of this test; and (7) protocol for the use of this test.

Progress: Sera from 53 cases of biopsy-proven primary hepatoma have been collected, and 47 cases (89%) are found to be positive for 5'-NPDase-V, whereas α-fetoprotein is positive (> 40 ng/ml) only in 22 cases (41.5%). Overall results show about 80% positive prediction for primary hepatoma also in these regions. 5'-NPDase-V has shown predictable value in lever metastasis, as demonstrated by intraoperative evaluations in 87 patients with colorectal cancer. Of the liver metastasis found in 19 patients at operation, 17 were previously found to be positive for this isozyme test. Liver metastasis was not found in 68 cases, but the test was positive in 27 cases and confirmed in 25 cases. In the remaining 41 cases when the test was negative, only 5 were confirmed to have liver metastasis in 6-12 months. This difference is statistically significant ($p < .001$). 5'-NPDase-V is also present in active HBsAg positive hepatitis and some late stage cirrhosis. However, there is a trend of increase 5'-nucleotide phosphodiesterase isozyme V/IV ratio in the following order: hepatitis < cirrhosis < hepatoma. This finding may lead to differential diagnosis of these diseases and support the alteration of gene expression as a basis for the appearance of 5'-NPDase-V.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 3/4/77

CONTRACT RESEARCH SUMMARY

Title: Experiment to Assess the Impact of Multi-site Screening on Total Cancer Mortality

Principal Investigator: Dr. Dean F. Davies
Name/Address University of Tennessee
Performing Organization: Memphis, TN

Contract Number: NO1-CB-53864
Starting Date: 6/23/75 Expiration Date: 12/22/77

Goal: The goal of this project is to achieve a design that can be put to clinical use in order to effectively reduce cancer mortality.

Approach: A team has been established with expertise in the fields of clinical oncology, radiology, epidemiology, and statistics. The experimental design will be developed on the basis of available knowledge of (1) high-risk factors, (2) quality of screening procedures, (3) variability in the effectiveness of present day treatment, (4) cost, (5) compliance with followup examinations and follow through procedures, (6) side effects of treatments, (7) incidence rates, (8) prevalence rates, and (9) case fatality rates. The rationale for the experimental design will be expressed in a format which makes explicit the use of available information and accepted assumptions.

Progress: In a community of a million persons, it seems reasonable to expect the experiment to demonstrate that the total cancer mortality of the 10-year survivors of the study population's second to sixth year of a six-year screening cycle will be approximatly 30% lower than that of the comparison population. Converted to the effect on the total cancer mortality, there would be a 7% decline in cancer deaths. At 1972 estimates of the cost of neoplasms to society, the estimated savings of this reduction would be at least $860 million dollars.

If the study group fails to demonstrate a significant decrease in total cancer mortality rate, it would be possible to estimate the total cost-effectiveness of the individual screening methods and to construct a cost-effective program.

Significance for Cancer Research: (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 5/4/76

CONTRACT RESEARCH SUMMARY

Title: Study of a Serum Alkaline Phosphatase Variant in Cancer Patients

Principal Investigator: Dr. Frank C. Larson
Name/Address University of Wisconsin
Performing Organization: Madison, WI

Contract Number: N01-CB-74173
Starting Date: 9/15/77 Expiration Date: 9/14/78

Goal: To define more clearly the possible diagnostic significance of the presence of an isoenzyme of alkaline phosphatase in serum of human cancer patients.

Approach: First, to screen a sufficient number of patients with a variety of cancers to determine whether the elevation in serum alkaline phosphatase variant (APV) found in preliminary studies is characteristic of all cancer types or restricted to cancers of certain organ sites and cell types. Next, to make sequential measurements in patients with cancer types which are found to be associated with APV to ascertain if variations in levels of serum APV reflect changes in cancer load following surgery, radiotherapy, or chemotherapy. Finally, to test effect of method improvement on discriminating power of the APV assay.

Progress: An isoenzyme of alkaline phosphatase has been discovered in the serum of patients who have a high degree of correlation with cancer. This isoenzyme (FHAP) is characterized by fast mobility in electrophoresis, sensitivity to homoarginine inhibition, resistance to phenylalanine inhibition and heat lability. Work so far under this contract has been directed toward improvement in the method and the establishment of the "normal control range." Normal persons (blood donors n=800) were found to have small amounts of the activity (median value 1.0 μ/l, 90%-10% separation point 2.0 μ/l.). There appears to be no sex difference, but slightly higher values may appear in older persons. Among cancer patients (N103) FHAP activity varied widely. Forty-three percent had values of below 2 units, 57% had values in excess of 2 units and 25% in excess of 5 units. The highest recorded value was 86 units. Closer examination has disclosed an apparent relationship with tumor load and degree of FHAP elevation. Most of the 43% who fell within the normal range were judged to be in complete clinical remission. Most of those 57% whose values were in excess of 2 units had progressive disease or were in relapse. APUD tumors do not appear to produce the isoenzyme.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: $54,000

CONTRACT RESEARCH SUMMARY

Title: Improvement of X-ray Imaging by Elimination of Scatter While Retaining the Resolution

Principal Investigator:	Dr. Melvin P. Siedband
Name/Address	University of Wisconsin
Performing Organization:	Madison, WI

Contract Number: NO1-CB-53914
Starting Date: 6/30/75 Expiration Date: 12/29/77

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a system that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical and/or electronic means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast, and dose reduction. Feasibility should be established during the first year of this contract.

Progress: 1) A servo-coupled paired slit system was used to generate basic data needed for the design of multiple slit systems. Tube loading limits were shown to be bounds on practical single paired slit systems. 2) A two-color cassette was developed for displaying energy absorption difference radiographs. 3) A two film differential absorption cassette was developed and shown to have greater energy differencing sensitivity than the two-color scheme. 4) A computer program and laboratory procedure were developed to improve the gain of the energy differencing systems. A side benefit of this study was the development of practical filters for the reduction of patient exposure in mammography, iodine contrast radiography (esp. cine-radiography), and high voltage chest radiography. Exposure reductions ranged from 35% to 75% due to filtration alone and up to 90% when coupled to other schemes (electrical filtration, etc.). Clinical evaluation is underway.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 78 Funds: 0 Site Visit Date: 4/21/76

CONTRACT RESEARCH SUMMARY

Title: Development of a Computerized Transaxial X-ray Reconstruction System

Principal Investigator:	Dr. Jay A. Stein
Name/Address	American Science and Engineering, Inc.
Performing Organization:	Cambridge, MA

Contract Number: N01-CB-53858
Starting Date: 6/30/75 Expiration Date: 5/1/78

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification, and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (N01-CB-53858 and N01-CB-53974). In the initial year, equipment and displays will be developed and fabricated for computerized transaxial X-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two years, clinical trials of equipment will be pursued on suitable human subjects, and results will be compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithm modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: The body scanner design configuration was completed during the first year, and fabrication of the unit was nearly completed by the end of the first year. Installation of the body scanner unit at Presbyterian Hospital, for evaluation under Contract N01-CB-53974, originally scheduled for the summer of 1976, was made during March and April 1977. Technical support for maintenance and optimization of the scanner was provided as it underwent clinical evaluation. The contract was terminated in April 1978, and the body scanner was left at Presbyterian Hospital for the continuing studies.

Significance to Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $223,000 Site Visit Dates: 5/5/76; 3/3/77

CONTRACT RESEARCH SUMMARY

Title: Standard Protocol for Evaluation of Imaging Techniques in Cancer Diagnosis

Principal Investigator: Name/Address Performing Organization:	Dr. John A. Swets Bolt, Beranek and Newman Cambridge, MA

Contract Number: N01-CB-64010
Starting Date: 6/10/76 Expiration Date: 12/9/78

Goal: To establish a new general protocol for comparison and evaluation of imaging techniques used in cancer diagnosis in order to contribute to the improved recognition of earlier cancer pathology.

Approach: The protocol will be developed to specify (1) the psychophysical procedures that provide unbiased measures of the diagnostic accuracy provided by each imaging technique, and (2) the data analyses that provide appropriate measures of the medical efficacy as well as the cost-benefit relationships associated with an imaging technique. To aid in the standard protocol development, a pilot study will be made involving an exploratory analysis of data from two collaborative studies sponsored by NCI. During the second year the protocol will be used to accomplish complete analysis of the two sets of NCI imaging data.

Progress: The standard protocol has been developed and has been tested in a pilot study. Because of unanticipated difficulty in obtaining necessary data required for the pilot study from the two collaborative studies, the pilot study of the standard protocol was delayed. Final revision of the standard protocol has been completed. Use of the standard protocol for comparison and evaluation of the imaging techniques studied in the two collaborative studies will be finished during 1978.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $74,612 Site Visit Date: 3/3/77

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Sadek K. Hilal
Name/Address Columbia-Presbyterian Medical Center
Performing Organization: New York, NY

Contract Number: N01-CB-43910
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide scanning, angiography and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of January 1977, 599 patients have been studied, including 468 Group I (213 Group IA, 132 Group 1B, and 123 Group 1C), 58 Group II, and 73 Group III.

Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up for two additional years. Analysis and interpretation of accumulated data are being actively pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $44,721 Site Visit Date: 2/18/75

CONTRACT RESEARCH SUMMARY

Title: Fabrication and Evaluation of Computerized Transaxial X-ray Reconstruction System

Principal Investigator: Dr. Sadek K. Hilal
Name/Address Columbia-Presbyterian Medical Center
Performing Organization: New York, NY

Contract Number: N01-CB-53974
Starting Date: 6/30/75 Expiration Date: 5/1/78

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (N01-CB-53858 and N01-CB-53974). In the initial year of the study, equipment and displays will be developed and fabricated for computerized transaxial X-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two-year endeavor, clinical trials of the equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithm modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: Algorithm evaluations have been carried out to test (1) detector fan of rays instead of a source fan; (2) tradeoffs in resolution between number of views and number of readings per view; (3) effect of polychromaticity on image quality; (4) production of lateral and frontal reconstructions; (5) filters, using patient data; (6) effect of decentering the body scanner. A protocol was established for clinical evaluation of the body scanner; the protocol was subdivided into body regions with separate physicians directly responsible for each region.

During winter 1976-1977, preparations were made for installation of the scanner when delivered by American Science and Engineering. Space renovation was completed in March 1977, the scanner was installed during March and April 1977, preliminary runs with the scanner began in May 1977, and clinical evaluations started soon thereafter. During the period of May 1977 to April 1978, when the contract was terminated, very satisfactory clinical performance was demonstrated for the scanner.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Date: 5/5/76

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Name/Address Performing Organization:	Dr. D. Gordon Potts Cornell University Medical School New York, NY

Contract Number: N01-CB-43980
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of April 1978, 574 subjects have been studied. This includes 444 patients in Group I (152 Group IA, 166 Group IB, and 126 Group IC), 56 patients in Group II, and 74 normal subjects. These patients were referred for study by the Departments of Neurology or Neurosurgery at New York Hospital and by Memorial Hospital.

Giant histological brain sections are being prepared on patients coming to autopsy to show the entire brain in the planes of the EMI sections; 121 cases have been studied in this manner. This will permit an accurate comparison of the EMI views with the other studies.

Patient enrollment has been completed and the only remaining work will be continuing patient follow-up for two additional years. Analysis and interpretation of accumulated data are being actively pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $55,778 Site Visit Date: 2/19/75

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Name/Address Performing Organization:	Dr. T. F. Budinger DOE -- Lawrence Berkeley Laboratory Berkeley, CA

Contract Number: Y01-CB-50304
Starting Date: 6/30/75 Expiration Date: 6/30/79

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for CT nuclide reconstruction based on transverse section emission data from human subjects are in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit location of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. Algorithms will be developed for computerized reconstruction of clinical data produced by transaxial nuclide projection machines. In addition, techniques for algorithm evaluation and comparison will be developed and applied. Algorithms, written in a high level programming language such as FORTRAN, together with phantom, animal and human emission and transmission projection data, and reconstructions will be recorded on nine track industry compatible magnetic tape and delivered to the NCI. The NCI shall make independent evaluations and disseminate this information to other investigators.

Progress: The final form of the User's Manual was completed in November 1977 and made available to interested workers. In contains 285 pages of descriptive material and complete FORTRAN listings with appropriate examples of each major algorithm and modifications. Work in the final year of the contract will concentrate on continued development of (1) a method for calculating dynamic functional images by computations in projection space; (2) a new general algorithm incorporating a priori information; (3) a new approach to the attenuation problem; (4) fan beam response characterizations; (5) a hardwired FFT design for interative reconstruction; and (6) additional development of the previously studied iterative hardwired reconstructor.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Date: 3/31/76

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Dr. Cornelius A. Tobias
Name/Address DOE -- Lawrence Berkeley Laboratory
Performing Organization: Berkeley, CA

Contract Number: Y01-CB-40302
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To evaluate the diagnostic potential of accelerated proton and various heavy ion beams in the detection of tissue abnormalities poorly or not detectable with X-rays; to develop techniques for the use of the particle beam of choice as a low dose adjunct to X-ray diagnostics.

Approach: With suitable phantoms and *in vivo* and *in vitro* biological specimens, the resolution and density detection sensitivity of various accelerated charged particle beams (H, He, C, O, Ne) will be compared using the LBL Bevatron/Bevalac for $Z \geq 2$ beams and the Harvard cyclotron for proton beams. In addition, the importance of beam energy and suitability of various detectors (such as plastic nuclear track detectors and photographic film) will be evaluated for the optimum beam particle-energy-detector system. Human diagnostic studies in cancer detection will be conducted with the system of choice. Comparisons of particle radiography with other noninvasive diagnostic techniques and pathology (when possible) will be made.

Progress: Studies to date, with phantoms and biological targets (including freshly excised mammary specimens), indicate that radiography with heavy ion beams (C, O, Ne) using a multi-layered stack of plastic foils for image registration affords substantially better lateral and depth resolution than light ion beams (proton, alpha) using a stack of photographic film. The heavy ion/plastics system produces superior depth resolution but inferior lateral resolution when compared with a conventional X-ray/film system. Techniques for low dose (0.1 rad) patient carbon mammography and extremity radiography have been developed and patients radiographed with promising results. Tumor structures in the human breast invisible to X-rays have now been seen with heavy ions. Imaging of the head and abdomen has also been studied in a few cases. Initial work in optical and computer synthesis of the images from a single multi-layered plastic stack into a single composite image has been done with encouraging results. During the final year of the contract work will concentrate on heavy ion imaging studies of (1) as many cancer patients as possible with emphasis on soft tissue; (2) isolated tissues and organs; and (3) micro-beam histological studies.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $106,400 Site Visit Date: 3/23/76

CONTRACT RESEARCH SUMMARY

Title: Development of Large Area Solid State Image Receptors for X-ray Imaging

Principal Investigator: Dr. Robert K. Swank
Name/Address General Electric Co.
Performing Organization: Schenectady, NY

Contract Number: NO1-CB-74112
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To improve clinical diagnostic radiology, for use in diagnosis of early cancer, by developing an X-ray sensor superior in clinical usefulness to those currently available.

Approach: A large area solid state X-ray detector will be produced which will transform the X-ray flux into a digitally stored high quality image. The system to be developed consists of two critical components, an X-ray absorbing electrophoretic image display cell and a laser scanning system, which are coupled with the necessary control, display, and image storage memory components. The critical components will be developed during the first year and their feasibility demonstrated. Further development and optimization of the critical components and the assembly of the remaining components into the finished system will be completed during the second year. Clinical evaluation of the system will be carried out during the third year.

Progress: Progress has been made during the first nine months of the contract toward production of the image display cell. Currently, a problem has developed concerning the sticking of electrophoretic pigment particles to the viewing surface of the image display cell. A solution to this problem, which produces a reduced contrast and image latency, must be found before the other areas of system design and development can be logically pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $750,260 Site Visit Dates: 6/16/77; 7/21/78

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator:	Dr. David O. Davis
Name/Address	George Washington University
Performing Organization:	Washington, DC

Contract Number: N01-CB-43981
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of April 1978, 525 patients have been studied; this number includes 376 in Group I (186 Group IA, 151 Group IB, 39 Group IC); 86 in Group II; and 63 normals (Group III). Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up for two additional years. Analysis and interpretation of accumulated data are being actively pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $34,513 Site Visit Date: 2/14/75

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques to Facilitate Passage of Colonoscope to the Cecum

Principal Investigator:	Dr. John A. Coller
Name/Address	Lahey Clinic Foundation
Performing Organization:	Boston, MA

Contract Number: N01-CB-74212
Starting Date: 9/26/77 Expiration Date: 9/25/78

Goal: To develop a means for improving early detection of small cancer lesions on endoscopically approachable surfaces of the colon in order to improve life expectancy of the postoperative patient.

Approach: A colonoscope advancing device will be developed to enhance the ease and speed of passage of currently available colonoscopes. Modeling studies and bench testing will be conducted during the initial year to determine which of several possible designs may be feasible. Further development of the colonoscope advancing device design will be carried out during the second year aided by physiological studies made to characterize as many physical parameters of the human colon as possible. Promising devices will be given preliminary clinical tests during the latter part of the second year. After further optimization the clinical prototype propulsive devices will be tested by clinical trials in the third year.

Progress: During the initial year good progress has been made in the model development and bench testing.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $105,000 Site Visit Dates: 7/15/77; 7/13/78

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator:	Dr. Paul F. J. New
Name/Address	Massachusetts General Hospital
Performing Organization:	Boston, MA

Contract Number: N01-CB-43983
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of May 1978, some 597 subjects have been studied, including 457 in Group I (82 Group IA, 121 Group IB, 254 Group IC), 79 in Group II, and 63 in Group III.

Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up for two additional years. Analysis and interpretation of accumulated data are being actively pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $23,734 Site Visit Date: 2/13/75

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Name/Address Performing Organization:	Dr. Hillier L. Baker, Jr. Mayo Foundation Rochester, MN

Contract Number: N01-CB-43982
Starting Date: 6/15/74 Expiration Date: 6/14/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of April 1978, a total of 634 patients have been included in the study: 451 Group I (304 Group IA, 86 Group IB, 61 Group IC), 82 Group II, and 101 Group III. .

Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up for two additional years. Analysis and interpretation of accumulated data are being actively pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $42,905 Site Visit Date: 1/16/75

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques to Facilitate Passage of Colonoscope to the Cecum

Principal Investigator: Dr. Max Epstein
Name/Address Northwestern University
Performing Organization: Evanston, IL

Contract Number: N01-CB-74114
Starting Date: 9/30/77 Expiration Date: 9/29/78

Goal: To develop a means for improving early detection of small cancer lesions on endoscopically approachable surfaces of the colon in order to improve the life expectancy of the postoperative patient.

Approach: A colonoscope advancing device will be developed to enhance the ease and speed of passage of currently available colonoscopes. Prototype design, fabrication, and preliminary testing with simulated environment models during the initial year will be followed by animal tests of promising designs during the second year. Further development and preliminary clinical testing during the second year will lead to the clinical prototype colonoscope advancing device which will be tested by clinical trials in the third year.

Progress: Initial work has progressed on design and fabrication of prototypes. A satisfactory method to fabricate the crawling sleeve design has been developed. Design of the body scope is progressing. An initial version of the vacuum anchor device has been constructed and will be tested soon. The inflatable liner device has been constructed and found to be effective. _In vivo_ animal testing of the liner will be accomplished shortly.

Significance For Cancer Research: (NCP Objective _5_ Approach _4_)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $100,000(est) Site Visit Dates: 6/9/77; 7/14/78

Title: Algorithms for Computerized Transaxial X-ray Reconstruction

Principal Investigator: Name/Address Performing Organization:	Dr. Gabor T. Herman Research Foundation, State University of New York Albany, NY

Contract Number: N01-CB-53860
Starting Date: 6/30/75 Expiration Date: 1/31/78

Goal: To improve detection and diagnosis of early cancer lesions by means of improved radiologic imaging techniques involving new mathematical algorithms for computerized transaxial X-ray reconstruction.

Approach: Algorithms will be developed for computerized three-dimensional reconstruction of X-ray absorption techniques from projection data collected via various sources and detector geometries. The reconstruction will minimize the anatomical and densitometric distortion and artifactual effects in regions of medical interest and decrease both scan time and radiation risk of the patient. Criteria also will be developed for evaluating efficacy of the algorithms. The algorithms will be written in a high level programming language such as FORTRAN and assembled in a library which takes advantage of input and output commonalities and which is modular and expandable. The library of algorithms plus suitable mathematical and actual test data and results will be written on nine track industry compatible tape and delivered to the National Cancer Institute for distribution to other investigators in the field.

Progress: An algorithm has been devised, implemented, and tested which can be used in a scanning device requiring less than 10 seconds scan time to detect lesions of 1 cm diameter or less in the chest or abdomen. Data base creation currently is in progress. A realistic detailed phantom of the human thorax has been produced along with simulated polychromatic X-ray projections for it. Subroutines have been written for most of the algorithms to be incorporated into the programming system and theoretical groundwork has been established for comparative evaluation of algorithms. Work has been completed on the required display program, and the library on tape was sent to NCI.

Significance for Cancer Research: (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Date: 3/30/76

CONTRACT RESEARCH SUMMARY

Title: Development of Ultrasonic Probes To Be Inserted Through Endoscopes for Use in Cancer Diagnosis

Principal Investigator:	Mr. Philip S. Green
Name/Address	SRI
Performing Organization:	Menlo Park, CA

Contract Number: N01-CB-74136
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To establish an ultrasonic system for improving early detection of small cancer lesions located in body regions approachable by endoscopes in order to improve life expectancy of the patient after appropriate treatment of the lesion.

Approach: The endoscopic ultrasonic detector system will be developed and fabricated during the first 21 months of the study by SRI personnel with clinical consultation by co-investigators from the School of Medicine of the University of California at San Francisco. Optimization of the system will be accomplished by the personnel from both organizations during the second year. Preliminary clinical evaluation and optimization of the system at UCSF will be done in the last quarter of the second year, and full clinical trials at UCSF will be conducted during the third year.

Progress: Preliminary work has been done on components of the detector system. Principal effort was directed toward development and evaluation of transducer arrays and of electronics for array control and signal processing. Mechanical design of the system was begun with particular emphasis on the bundle of coaxial cabler which must be integrated with the endoscope.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $397,764 (estimate) Site Visit Dates: 6/7/77; 6/22/78

CONTRACT RESEARCH SUMMARY

Title: Radioisotope Surface Markers and Detectors for Endoscopic Techniques

Principal Investigator:	Dr. James M. Woolfenden
Name/Address	University of Arizona Medical Center
Performing Organization:	Tucson, AZ

Contract Number: N01-CB-64012
Starting Date: 6/30/76 Expiration Date: 6/30/79

Goal: To establish a means for improving early detection of small cancer lesions on endoscopically approachable surfaces in order to improve the life expectancy of the patient.

Approach: A system will be developed for endoscopic detection of small cancer lesions in mucosal surfaces by means of tumor-seeking radioisotopic markers. The system will include one or more appropriate tumor-seeking radioisotopically labeled markers and a detector system suitably matched to the emission characteristics of the markers to provide optimum localization of lesions.

During the first year the work will concentrate on development and testing of the tumor-seeking markers. During the second year development work on the tumor-seeking markers will continue, and the testing of the markers in the animal models will be expanded. In addition, the work will be started to develop suitable detectors.

During the third year any necessary additional development or modification of the radiolabeled tumor-seeking agents will be made; development and optimization of the detector system and its incorporation into endoscopic equipment will continue; and clinical evaluation of the system using a previously agreed upon protocol will be conducted.

Progress: Radiolabeled bleomycin has been concentrated on for the tumor-seeking marker. Separation of the individual cobalt labeled bleomycin fractions by means of high pressure liquid chromatography is being studied. Separate fractions are being studied for relative distribution in animal tumor models and are being characterized by thin layer chromatography and high voltage electrophoresis. A cadmium telluride detector is being characterized, and other detectors are expected including sodium iodide and mercury iodide. A tumor phantom apparatus has been constructed and is being used to evaluate detector performance.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Dates: 11/5/76; 10/28/77

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator: Dr. Wayne A. Tompkins
Name/Address University of Illinois
Performing Organization: College of Veterinary Medicine
Urbana, IL

Contract Number: N01-CB-64073
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year, CEA and TSA (tumor-specific antigen) will be isolated from human tumors, purified, and injected into animals for antibody production. The resulting antibodies, after separation and purification, will be tested *in vitro* and in suitable *in vivo* animal models. Concurrently, development of antibody labeling techniques will be started and growth of intestinal carcinoma cells initiated in nude mice.

During the second year, the CEA and TSA will be produced in larger amounts, labeled, and tested *in vivo* in animals.

Progress: Both primary and metastatic GI-associated tumor material is being collected; this includes paracentesis fluid, liver tissue, and primary colon lesions. Isolation and purification of CEA from this material are proceeding, and animals have been injected with some of that material to begin anti-CEA immunoglobulin production. ^{51}Cr cytotoxicity studies have been started. A solid phase ^{131}I-anti-CEA binding test has been developed for quantitating CEA on the surface of HCT-8R cells. Preliminary work has been done on extractions of TSA from colon tumor tissue. Work from the present until the contract is terminated in June 1979 will concentrate on: continued production of CEA and anti-CEA and purification by immunoabsorbant column elution; development of an *in vivo* system for tumor localization of ^{125}I-anti-CEA; and further purification and evaluation of PCA-S2 as a tumor-associated antigen.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: 0 Site Visit Dates: 9/24/76; 3/30/77

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator: Dr. David M. Goldenberg
Name/Address University of Kentucky Research Foundation
Performing Organization: Lexington, KY

Contract Number: N01-CB-64011
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year the tumor localizing efficiency of ^{131}I-labeled anti-CEA immunoglobulins will be evaluated in animal studies. After their suitability is established by the animal studies, the materials will be tested in human subjects. The clinical testing may begin before the end of the first year and will be done first with advanced incurable cancer patients, then proceed to those with curable cancer, and finally, if the initial studies are successful, done with those having questionable cancer. Other radiolocalization systems based upon non-CEA tumor antigens will also be studied concurrently with those described above. However, since these systems are in earlier stages of development, no clinical trials will be made on them before the third year of the study.

Progress: ^{131}I-labeled anti-CEA immunoglobulin has been purified and tested for purity and suitability for human use. First clinical testing of the material was begun during the summer of 1977, and by May 1978 approximately 70 cancer patients will have been studied. Preliminary results are encouraging. After computer subtraction of blood pool background activity, localization of the labeled immunoglobulin could be detected, at 48 hr post-injection, in most of the cases studied. CEA-containing tumors at various body sites could be localized by external scintillation techniques. Scanning results were negative in patients without demonstrable tumors or with tumors apparently free of CEA.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 78 Funds: $186,741 Site Visit Dates: 9/23/76; 10/27/77

CONTRACT RESEARCH SUMMARY

Title: Development of Large Area Solid State Image Receptors for X-Ray Imaging

Principal Investigator: Name/Address Performing Organization:	Dr. Moshe Ein-Gal Xerox Corporation Pasadena, CA

Contract Number: NO1-CB-74211

Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To improve clinical diagnostic radiology, for use in diagnosis of early cancer, by developing an X-ray sensor superior in clinical usefulness to those currently available.

Approach: A large area solid state X-ray detector will be produced which will transform the X-ray flux into a digitally stored high quality image. The system to be developed, an X-ray selenium electronic linear scanner, will use the capability of a selenium alloy photoreceptor to create a latent image from absorbed X-rays. The latent image, an array of electric charges on the photoreceptor surface, will be detected by a microelectrometer scanning arrangement. Signals from the electrometer will be coupled with the necessary control, display and image storage memory components. Feasibility of the imaging system components will be developed in the first year. Further design, development, and optimization of the system will be completed during the second year. Clinical evaluation of the total imaging system will be conducted during the third year.

Progress: Preliminary results in producing the imaging system components are encouraging.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.

Program: Diagnosis

Technical Review Group: Diagnostic Radiology Committee

FY 78 Funds: $699,758(est) Site Visit Date: 6/6/77; 6/23/78

CONTRACT RESEARCH SUMMARY

Title: Development of New Methods of Single Cell Separation

Principal Investigator:	E. Robert Schildkraut
Name/Address	Block Engineering, Inc.
Performing Organization:	Cambridge, MA

Contract Number: N01-CB-74095
Starting Date: 8/17/77 Expiration Date: 8/16/79

Goal: Development and testing of a single cell separator that will reliably associate individual separated cells with specific quantitative measurements.

Approach: The contractor shall develop and analyze techniques for deposition, fixation, staining, and scribing system for gynecologic cells on film. The speed and fluid volumes will be compatible with the output of a flow microfluorometric system. The design of this scribing system will not be such as to preclude its adaptation as a module to presently available FMF systems other than the contractor's. The contractor shall also develop and analyze a fiducial system which will allow reliable unique recall and identification of individual cells.

Progress: The engineering and some biological problems regarding the scribe, the proper selection of the film, proper layering of fluid on film, and film strip drying and staining have been resolved. A module is undergoing testing and further fabrication.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
The development of a reliable clinical screening system depends on the identification and assurance that "abnormal" quantitative signals are associated with abnormal cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Cytology Automation Committee
FY 78 Funds: $322,882 Site Visit Date: 2/9/77, 3/6/78

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers Useful for Gynecologic Specimens

Principal Investigator:	Dr. Brian H. Mayall
Name/Address	DOE - Lawrence Livermore Laboratory
Performing Organization:	Livermore, CA

Contract Number: Y01-CB-40300
Starting Date: 6/1/74 Expiration Date: 6/29/79

Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: The contractor will continue to implement and evaluate the dual laser sorter. The development and evaluation of new markers for automation of clinical cytology will continue. Evaluation will be carried out on the dual laser sorter. Computer controlled sorting will be added to the dual laser system. Subpopulation of clinical cytology specimens from normal to premalignant and malignant cases will be identified using new staining and single and multiparameter analysis techniques. These subpopulations will be sorted and analyzed by CYDAC after morphologic characterization.

Progress: A new specimen preparation procedure has been developed which reduces "false alarm" signals from degenerating leukocytes. New cytochemical probes have been examined for dual staining procedures for DNA, for measurement of fluorescent energy transfer and for application of these procedures to gynecologic cytology specimens. The dual laser sorter has been constructed and is being evaluated.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3) Development and evaluation of techniques to rapidly screen cell populations for premalignant and malignant cells.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $292,041 Site Visit Date: 5/14/76, 9/1/77

CONTRACT RESEARCH SUMMARY

Title: Application of Flow System Methods of Cell Analysis and Sorting to Cancer Screening

Principal Investigator:	Dr. Paul Mullaney
Name/Address	DOE - Los Alamos Scientific Laboratory
Performing Organization:	Los Alamos, NM

Contract Number: Y01-CB-60311
Starting Date: 4/1/71 Expiration Date: 5/31/79

Goal: Automation of clinical cytology screening and diagnosis.

Approach: The contractor shall (1) continue the development and evaluation of the parabolic flow chamber, (2) continue fabrication and evaluation of the two laser cell analysis and sorting system including measurements of cells stained with two or more fluorochromes, two-color light scattering measurements and Coulter volume, (3) develop protocols for new fluorochromes using model cell systems and clinical material.

Progress: The contractor has obtained a large number of gynecologic specimens for which some dispersal, fixation, and staining techniques have been developed. The acriflavine-feulgen procedure for DNA staining, mithramycin as a rapid DNA stain, propidium iodide and fluorescein isothiocyanate as a simultaneous DNA-protein staining technique, narrow angle light scatter, multi-angle light scatter, time-of-flight, and Coulter volume parameters have been implemented on flow systems. Studies have been carried out on the effect of cell orientation on light scattering signatures of mammalian cells and on evaluation of the acquired data. The two laser cell analysis and sorting instrument have been fabricated. A modular data acquisition system is being designed and implemented.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Cytology Automation Committee
FY 78 Funds: $291,102 Site Visit Date: 1/5/77

CONTRACT RESEARCH SUMMARY

Title: Vaginal-Cervical Sample Sources

Principal Investigator: Name/Address Performing Organization:	Dr. Gregorio Delgado Georgetown University Hospital Washington, DC

Contract Number: N01-CB-70482
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 1050 gynecologic cytopathology samples obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate instrumentation and methods of sample preparation and staining, both at NCI and at other contracting institutions within this program.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging from normal to invasive squamous cell carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $42,000

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. Laure Aurelian Johns Hopkins University School of Medicine Baltimore, MD

Contract Number: N01-CB-74170
Starting Date: 7/18/77 Expiration Date: 7/17/79

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate HSV antigens: (i) Total viral antigens, (ii) AG-e and (iii) fractions of AG-e, and prepare corresponding purified antisera to test sensitivity and specificity of these antisera to human gynecologic materials. Immunological and biochemical characterization of the antigens will be done.

Progress: The preparation of antigens from HSV infected cells and the purification of the antigens have proceeded as scheduled. Testing by indirect immunofluorescent staining appears promising. Many more specimens and cells will be studied.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3) The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $79,500 Site Visit Date: 2/24/77

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth Castleman JPL - California Institute of Technology Pasadena, CA

Contract Number: Y01-CB-70314
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall determine whether or not current state of the art in pattern recognition is adequate to support the development of an economically viable cytological screening instrument based on single cell classification. Specimen preparation protocol will be optimized so as to produce acceptable single cells for digitization. A large number of digitized images will be accumulated in the library for subsequent feature extraction program to produce a feature data base. A series of classification experiments will be run on the feature data base to select the subsets of cell measurements that yield the best overall performance.

Progress: Specimen preparation protocol has been completed. Digitized cell images are being acquired and classification algorithms are being developed.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Cytology Automation Committee
FY 78 Funds: $200,862 Site Visit Date: 5/23/77

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Markers for Gynecologic Specimens

Principal Investigator: Dr. Torbjorn Caspersson
Name/Address Karolinska Institute
Performing Organization: Stockholm, Sweden

Contract Number: N01-CB-43945
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: Automation of clinical cytologic screening and diagnosis, concomitant with improvement in diagnostic accuracy and throughput.

Approach: Dr. Caspersson has pioneered the development of innovative methods of investigative cytology and cytogenetics. Under this contract, he will develop and evaluate prototype machines for automatically measuring cytologic features of clinical gynecologic specimens using high resolution scanning microscopy with absorption ultraviolet-spectrophotometry, interferometry and cytofluorometry, individually and in combination. Quantitative measurements of the various cell types of the female genital tract will yield multiparameter data which can be used as discriminators for these different cell types by automated cytology instruments.

Progress: A two-parameter instrument has been constructed. The instrument consists of a computer-controlled servomotor driven scanning microscope, a micro- spectrophotometer, and a microinterferometer. The scanning microscope is used to examine and select cells and has the capability to recall the previously chosen cells for reexamination and analysis. The integrated instrument permits microspectrophotometric measurements in the UV and/or visible range, followed by microinterferometric measurements on the same cell while the cell remains in the same microscope field of vision. Modification and improvement of the systems including incorporation of image intensifier-camera system have been completed. Data on gynecologic materials are being accumulated and analyzed.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
Development of very rapid high resolution multiple modality automated scanning microscopes will advance image processing approaches for cytologic screening and diagnosis, and promote better understanding of normal, precancerous and cancerous states.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: 0 Site Visit Date: 6/26/76

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Staining of Tissues and Exfoliated Cells

Principal Investigator: Name/Address Performing Organization:	Dr. Ralph D. Lillie Louisiana State University Baton Rouge, LA

Contract Number: N01-CB-43912
Starting Date: 1/11/74 Expiration Date: 1/10/79

Goal: Development of hematoxylin substitutes for diagnostic staining of tissues and exfoliative cells.

Approach: (1) Synthesize various classes of mordant dyes or obtain same from either specific manufacturers or from the archives of the Biological Stain Commission. (2) Perform studies on various tissues with combinations of dyes obtained to achieve staining in accordance with criteria satisfactory to the Project·Officer and to the Biological Stain Commission's certification criteria. (3) Procure samples of possibly suitable mordant dyes offering blue or black mordant colors which may be suitable to replace hematoxylin in one or more of its histological staining uses. (4) Subject such samples to the usual tests applied by the Biological Stain Commission for the certification of hematoxylin. (5) Apply favorable test results to routine surgical diagnostic material, in Papanicolaou smear diagnosis, and in other staining procedures applicable to the differential diagnosis of various cancers, evaluating results in each case in comparison of previously standard hematoxylin procedures and with the best of the previously proposed substitutes.

Progress: Synthesis and testing are on schedule as outlined above.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
Stain development is essential to the cytological diagnosis of cancer.

Project Officer: M. F. Stanton, M.D.
Program: Cytology Automation
Technical Review Group: Ad Hoc Committee Laboratory of Pathology
FY 78 Funds: $13,500

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. John J. Docherty Pennsylvania State University University Park, PA

Contract Number: N01-CB-74171
Starting Date: 7/18/77 Expiration Date: 7/17/79

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate "tumor antigen" extracted from HSV-1 and HSV-2 transformed cells, and prepare corresponding purified antisera for labeling with peroxidase and fluorescein isothiocyanate and testing on gynecologic exfoliated cervical cells and biopsy specimens. Cell by cell analysis will be done to assess the sensitivity and specificity of the assay. Techniques for immunofluorescent staining of exfoliated cells in suspension will be developed for use with flow microfluorometric cell sorter.

Progress: Exfoliated cells are being used to test the sensitivity and specificity of the isolated "tumor antigen." The cells are subsequently destained and restained by the "Pap" procedure for diagnosis.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. James W. Bacus Rush-Presbyterian-St. Luke's Medical Center Chicago, IL

Contract Number: N01-CB-74190
Starting Date: 9/30/77 Expiration Date: 9/2/80

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall conduct a comprehensive study to determine single cell vs. specimen classification accuracies for image processing algorithms and for cytotechnologist. This will determine how well the machine algorithms are performing. Five experimental tasks will be done: (1) sample acquisition, (2) cell acquisition, (3) observer recognition, (4) cell classification algorithms development, and (5) analysis and evaluation of results.

Progress: Patient selection and sample acquisition is close to completion. Fabrication of the cell acquisition system is well underway. Software is being written for the various aspects of the cell acquisition protocol with emphasis on scene segmentation methods.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3) Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Cytology Automation Committee
FY 78 Funds: $330,000 Site Visit Date: 5/30/77

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Preparations of Cytology Specimens

Principal Investigator: Name/Address Performing Organization:	Dr. Walter E. Tolles State University of New York Brooklyn, NY

Contract Number: N01-CB-43955
Starting Date: 6/1/74 Expiration Date: 5/31/79

Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automation, and to supply NCI and other contractors with treated and untreated gynecologic samples for use in the evaluation of sample preparation and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology or other contractors at least 12 specimens per week ranging in classification from normal to invasive carcinoma. These specimens will be either untreated or disaggregated by ultrasound.

Progress: Ultrasound appears to be the most effective means of cell disaggregation for this contractor. An improved ultrasonicator has been developed so the energy output can be monitored. A new sedimentation chamber has been designed and tested. Other aids to sample preparation have been evaluated, such as automatic slide making and nephelometry which gives an indication of the cellular content of the samples before processing. The gynecologic cytology samples which have been delivered to NCI have been adequate in diagnostic cellular material. A portion of these samples has been sent to other contractors for use in their studies.

Significance for Cancer Research: (NCP Objective 5 Approach 5.4)

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $45,028 Site Visit Date: 11/19/75

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. Parviz Hanjani Temple University Medical School and Hospital Philadelphia, PA

Contract Number: 263-77-C-0513
Starting Date: 6/20/74 Expiration Date: 6/30/79

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 600 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging in diagnosis from normal through invasive squamous cell carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $24,000 Site Visit Date: 7/11/74

CONTRACT RESEARCH SUMMARY

Title: Image Processing for Development of Automated Cell Recognition System

Principal Investigator: Dr. George L. Wied
Name/Address University of Chicago
Performing Organization: Chicago, IL

Contract Number: N01-CB-33873
Starting Date: 5/31/73 Expiration Date: 4/30/81

Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution processing-based decision making.

Approach: The contractor shall evaluate information requirements for characterization and recognition of normal, premalignant and malignant cells from the human female genital tract by means of digital image processing.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Sorted cleaned cell images are available for other contractors to analyze with their own algorithms. Further details on scanned cells are being done using 3-color scanner. A new computer program has been written to evaluate the new information. The multi-spectral band scanner has enriched the data base for cell image processing. The new color features enhance the classification of metaplasias, dysplasias, and malignant cases. Feature extraction for chromatin distribution patterns has been implemented. High-resolution data acquisition module and cell detection module hardware are being implemented. An integrated system is being tested using the best and optimal algorithms to determine the classification performance of the system to classify cells as normal and non-normal.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
A rapid, high-resolution, multi-spectral digital scanner for clinical gynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the populations at large and at high risk.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $196,519 Site Visit Date: 6/8/76, 9/8/77

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Name/Address Performing Organization:	Dr. Harold Haines University of Miami School of Medicine Miami, FL

Contract Number: N01-CB-74096
Starting Date: 5/13/77 Expiration Date: 5/12/79

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: Contractor shall isolate and purify cervical carcinoma antigen (CCA). Appropriate antisera to CCA will be prepared and cell by cell analysis using indirect immunofluorescent technique and restaining by Pap staining procedure will be done. Exfoliated cells, tissue imprints and tissue sections will be used. Technique for immunofluorescent staining of exfoliated cells in suspension will be developed for use in flow microfluorometric cell sorter.

Progress: Crude and purified CCA have been accumulated and corresponding antisera obtained. Exfoliated gynecologic specimens have been tested. Further purification and characterization of the antigen and additional cell-by-cell analysis are being pursued.

Significance for Cancer Research: (NCP Objective 5 Approach 5.3)
The development of new markers will improve the prosects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $58,025

CONTRACT RESEARCH SUMMARY

Title: Slit-Scan Technique as Cancer Prescreening Automated System in Cytology

Principal Investigator:	Dr. Leon L. Wheeless, Jr.
Name/Address	University of Rochester
Performing Organization:	Rochester, NY

Contract Number: N01-CB-33862
Starting Date: 3/19/73 Expiration Date: 3/18/79

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The contractor will construct and test the X-Y-Z Slit Scan Flow System. A benchmark study of the X-Y-Z system will be performed to 1) determine system characteristics including rate and causes of remaining false alarms and 2) document true alarm rates for abnormal specimens. Correlation studies will continue and studies on second stage processing techniques will begin.

Progress: The contractor has evaluated the VIDICON imaging system and additional causes of false alarms have been determined. Classification algorithms were modified to eliminate some of the false alarms. The X-Y-Z Slit Scan Flow System was designed to eliminate the remaining false alarms and is being fabricated. Sample preparation protocols are continuing to be evaluated.

Significance for Cancer Research: (NCP Objective 5 Approach 5.4) The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed, and availability in screening and diagnosis of cancerous and precancerous lesions.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 78 Funds: $357,062 Site Visit Date: 6/23/76, 1/12/78

CONTRACT RESEARCH SUMMARY

Title: Pre-clinical Studies of Chemotherapeutic Studies for Mycosis Fungoides

Principal Investigator: Name/Address Performing Organization:	Dr. R. M. Folk Battelle Memorial Institute Columbus, OH

Contract Number: N01-CM-43746
Starting Date: 3/1/75 Expiration Date: 9/28/80

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: To increase the knowledge of the pharmacology of chemotherapeutic agents in topical application, investigation of cutaneous irritation, and percutaneous absorption will be carried out.

Progress: Subcontract has been awarded to the University of Mississippi School of Pharmacy. Cutaneous irritation studies have been done. Reports to date indicate that a few chemotherapeutic agents are irritating to rabbit skin after a single application. Initial percutaneous absorption studies have been completed; testing of additional radio-labeled drugs will begin shortly.

Significance for Cancer Research: (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $74,644

CONTRACT RESEARCH SUMMARY

Title: A Screening System for Topical Chemotherapy of Mycosis Fungoides

Principal Investigator:	Dr. Stanford Lamberg
Name/Address	Johns Hopkins University
Performing Organization:	Baltimore, MD

Contract Number: N01-CB-63927
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: Chemotherapeutic agents are obtained commercially and from the DCT, NCI, and these agents are being screened by clinical patch testing in patients with mycosis fungoides.

Progress: Investigative new drug applications have been obtained from the FDA and patch test screening of these agents has begun at Hopkins and other affiliated institutions comprising the Mycosis Fungoides National Cooperative Group. More widespread testing of agents found to be active by patch test screening will begin shortly.

Significance for Cancer Research: (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Tumor Virus Expression in Normal and Transformed Cells

Principal Investigator:	Dr. Kenneth Blackman
Name/Address	Meloy Laboratories, Inc.
Performing Organization:	Rockville, MD

Contract Number: N01-CB-74139

Starting Date: 6/15/77 Expiration Date: 6/14/79

Goal: To study expression of tumor viruses in tissue culture and in animals.

Approach: Viral biochemical, serologic, and biological parameters are monitored in different systems to define the regulatory mechanisms of tumor virus expression and to relate the expression to tumor production.

Progress: A quantitative assay for transfection of mammalian leukemia and sarcoma viruses has been developed for use in studies of control of viral expression.

Infectious unintegrated Harvey sarcoma virus DNA has been found to be a double stranded molecule of about 4×10^6 daltons. Sarcoma viral DNA can transform in the absence of helper viral genomes. Also, bulk cellular DNA from normal AKR mouse cells, which is not infectious under standard transfection conditions, is infectious if recipient cells are treated with 5-iododeoxyuridine. This procedure may be useful in establishing the infectious potential of other endogenous viral genomes.

In addition, glucocorticoids have been found to induce foci of morphologically transformed cells in a morphologically untransformed mink cell line which contains the Moloney murine sarcoma viral genome. The continued presence of glucocorticoid is required for the maintenance of foci. The induction of foci correlates with intracellular increases in sarcoma virus-specific viral RNA and in murine viral p30 protein.

Significance for Cancer Research: (NCP Objective 6 Approach 2)

Project Officer: Dr. Douglas R. Lowy

Program: Immunology Support

Technical Review Group: Ad Hoc Committee

FY 78 Funds: $85,350

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Name/Address Performing Organization:	Mr. W. Bruce Ramsay Computer Services Division National Bureau of Standards Gaithersburg, MD

Contract Number: Y01-CB-80047

Starting Date: 7/1/73

Expiration Date: 9/30/78

Goal: To provide computer facility for mathematical computation related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Name/Address Performing Organization:	Mr. Wayne H. Anderson USAARDC Aberdeen Proving Ground, MD

Contract Number: Y01-CB-80315

Starting Date: 5/1/78

Expiration Date: 9/30/78

Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $2,000

CONTRACT RESEARCH SUMMARY

Title: Preparation and Analysis of Cell Surface Protein (CSP) Fractions

Principal Investigator:	Dr. David Schlesinger
Name/Address	University of Illinois Medical Center
Performing Organization:	Chicago, IL

Contract Number: N01-CB-74214
Starting Date: 9/30/78
Expiration Date: 9/30/79

Goal: To prepare and analyze the structure of cell surface protein.

Approach: Cell surface protein has been treated by cyanylation to prepare fragments. Six fragments were obtained which should be useful for sequence studies. These have been tested for adhesive activity and are inactive. CSP has also been cleaved with *S. aureus* protease. Surprisingly only 10% of the available residues were hydrolyzed. A large peptide that is N-terminal has been tentatively identified.

CSP has also been cleaved with cyanogen bromide. One of the fragments so obtained may contain the disulfide cross-link area.

CSP has been shown to be absent in many kinds of cancer cells and its absence has been correlated with the tumorigenicity of such cells. The knowledge of the structure of CSP will further our understanding of the cancer process.

He will further characterize the CSP fragments obtained by the above method.

Significance for Cancer Research: (NCP Objective 2 Approach 3)

Project Officer: Dr. Ira Pastan
Program: Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay of Immunoglobulin Molecules

Principal Investigator: Name/Address Performing Organization:	Dr. Kenneth Kortwright Meloy Laboratories, Inc. Springfield, VA

Contract Number: N01-CB-63932
Starting Date: 3/15/78 Expiration Date: 3/14/79

Goal: To perform radioimmunoassays of immunoglobulin molecules IgM, IgA, IgG, IgD, and IgE in lymphocyte culture supernatants or in other biological fluids.

Approach: The contractor is to perform determinations of human IgG, IgA, IgM, IgD, and IgE, and mouse IgA and IgM by double antibody radioimmunoassay by procedures defined by the project officer and using reagents supplied by the project officer. This contract provides critically required research support for studies on the nature of immunodeficiencies that are associated with a high incidence of malignancy and on the cause of the immunodeficiency associated with malignancies of the B cell or plasma cell system. In addition, these studies are directed at defining retained immunological capabilities of T cell leukemias.

Progress: The contract has established radioimmunoassays for IgG, IgA, IgM, and IgE of man for IgA and IgM of the mouse. These assays have been used to quantitate the rate of immunoglobulin synthesis by pokeweed mitogen stimulated, peripheral blood lymphocytes of man or of splenic lymphocytes of the mouse in *in vitro* cultures. Patients with a T cell leukemia associated with the Sezary syndrome have been shown to have a malignant expansion of helper T cells whereas a patient with acute lymphocytic leukemia was shown to have a malignancy of the suppressor T cells. A subset of patients with common variable hypogammaglobulinemia has been shown to have excessive numbers of suppressor T cells that inhibit gammaglobulin synthesis by B cells. Certain patients with selective IgA deficiency have been shown to have an IgA class specific suppressor. Patients with multiple myeloma have hypogammaglobulinemia due in part to excessive numbers of suppressor macrophages. These studies are defining the nature of disorders of the immune system that led to a high incidence of cancer. In addition, they provide information on the cause of immunodeficiency that arises secondary to certain forms of malignancy. Finally, these studies have provided insights into the retained functions of T cell leukemias.

Significance for Cancer Research: (NCP Objective 3 Approach 4)

Project Officer: Thomas A. Waldmann, M.D.
Program: Biology Support
Technical Review Group: Ad Hoc Review Group
FY 78 Funds: $136,755

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia

Principal Investigator: Dr. John C. Harshbarger
Name/Address The Smithsonian Institution
Performing Organization: Registry of Tumors in Lower Animals
Washington, DC

Contract Number: N01-CB-33874
Starting Date: 7/1/73 Expiration Date: 6/30/79

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: The principal investigator directs the operation of a registry of tumors in lower animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made. The Registry also serves in a consulting capacity to other agencies concerned with diseases in lower animals, such as the Environmental Protection Agency, the U.S. Bureau of Fisheries (Commerce Dept.), and the Environmental Health Center.

Progress: Specimen accessions during the past year numbered 239, bringing the total collection to 1,837. Fifty-two percent of the new accessions are neoplasms, the majority from mollusks, teleost fish, amphibians, and reptiles. While lesions that appear to be neoplastic by morphological criteria continue to be found in mollusks and arthropods (insects alone represented), the other invertebrate phyla appear to have no representation except for lesions of controversial nature. Types of tumors accessioned are listed in the 1977 Progress Report of the Registry. The world literature collection expanded by some 360 papers, bringing the total to 3,510. Seven original reports and reviews were published by the Director during the 1977 fiscal year, or are in press.

Significance for Cancer Research: Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.

Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $135,818 Site Visit Date: 5/12/77

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator:	Andrew S. Tegeris
Name/Address	Pharmacopathics Research Laboratories
Performing Organization:	Laurel, MD

Contract Number: N01-CB-33887
Starting Date: 7/1/73 Expiration Date: 12/30/78

Goal: To maintain and observe animals on experiments designed to develop the means to reduce the effectiveness of external agents for producing cancer with a variety of types of materials, particularly mineral fibers and tobacco products.

Approach: Improve animal models of human cancer and evaluate effects of combined factors by assessing the response of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: We are currently testing carcinogenic response to many types of durable fibers in the pleura of the rat. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers. Tobacco smoke fractions and air pollutant fractions are being evaluated for carcinogenicity on a quantitative level.

Significance for Cancer Research: Animal models of human cancer are essential to identifying causative agents, mechanisms of action and therapeutic trials.

Project Officer: Mearl F. Stanton, M.D.
Program: Biology Support
Technical Review Group: Ad Hoc Relevance Review Group
FY 78 Funds: $100,000 (6 months) Site Visit Date: Monthly

CONTRACT RESEARCH SUMMARY

Title: Characterization of a Transformation-Sensitive Cell Surface Glycoprotein

Principal Investigator: Dr. Frank Holly
Name/Address Eye Research Institute of Retina
Performing Organization: Foundation
Boston, MA

Contract Number: N01-CB-74209
Starting Date: 9/30/78 Expiration Date: 9/30/79

Goal: To elucidate the mechanism of action of the adhesive, transformation-sensitive glycoprotein CSP by surface chemical analysis.

Approach: CSP is an adhesive glycoprotein that is generally decreased after malignant transformation. It is normally self-associated in aggregates and fibrils. Surface chemical analyses will permit highly sensitive investigations of CSP in this self-associated form to complement standard biochemical approaches that require solubilization of CSP. CSP is routinely spread as a monomolecular layer at an air-liquid interface in a Langmuir trough, and the film pressure-area isotherm is determined. The specific area of CSP is found to be 12,000 Å at physiological pH when spread at an interface, compared to 150 Å when adsorbed; this indicates a highly asymmetric shape with an axial asymmetry of 80:1.

CSP's specific area is found to be highly dependent on ionic strength and pH, as is its biological activity. Interestingly, removal of calcium by EGTA results in an altered specific area; this is the first physical confirmation of the structural calcium requirement first suggested by biological assays. In other work, a new theory for cell adhesion has been developed that takes into account contributions of the glycocalyx to adhesive interactions (Dolowy and Holly, Theor. Biol., in press). Future work will include dynamic measurements of CSP interactions using a surface viscometer now under construction, comparisons of CSP with chemically inactivated CSP or a related adhesive protein "cold insoluble globulin"; and use of ellipsometry to study the effects of varying the thickness of substratum-adsorbed CSP on its surface chemical properties.

Significance for Cancer Research: (NCP Objective 2 Approach 3)

Project Officer: Dr. Kenneth Yamada
Program: Biology Support
Technical Review Group: Ad Hoc Committee
FY 78 Funds: $82,000

CONTRACT RESEARCH SUMMARY

Title: Administrative Support Services

Principal Investigator: Name/Address Performing Organization:	Ms. J. Aldridge Kappa Systems, Inc. Arlington, VA

Contract Number: N01-CB-53866
Starting Date: 11/1/74 Expiration Date: 10/31/77

Goal: To support administrative staff in preparation of scientific meetings and the research components in accomplishing their administrative tasks.

Approach: Current staffing situation places impossible demands on the administrative staff and committee chairmen (presently non-NCI scientists) in carrying out their responsibilities. This project is to support such routine tasks.

Progress: Executive secretaries and senior staff members were assisted in organizing scientific meetings of various committees. This made it possible to handle effectively the volume of administrative burden without overstraining available resources. Committee meetings and three program overviews were organized during the contract year. On the intramural research level, routine administrative and support activities were handled with minimal disruption and innovative research. In particular, the contractor assisted the intramural staff in handling reprint service and in preparation of manuscripts.

Supportive background materials were prepared for: Diagnosis Research Overview Immunotherapy Conference and BCTC Report to the Profession - the "White House" conference. Assistance was rendered in preparation of the Annual Report for the Division. The contract terminated in FY 78.

Significance for Cancer Research: (NCP Objective 5 Approach 1-7)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: DCBD
Technical Review Group: Ad Hoc Committee
FY 78 Funds: 0 Site Visit Date: 9/74

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